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A COMPUTER PROGRAM TO PROCESS SPECTROPHOTOMETRIC ANALYTICAL DAT--ETC(U)
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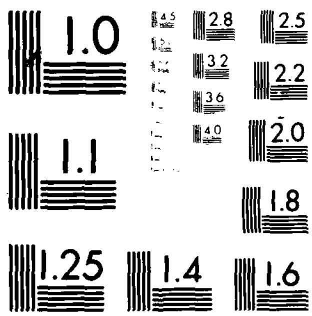

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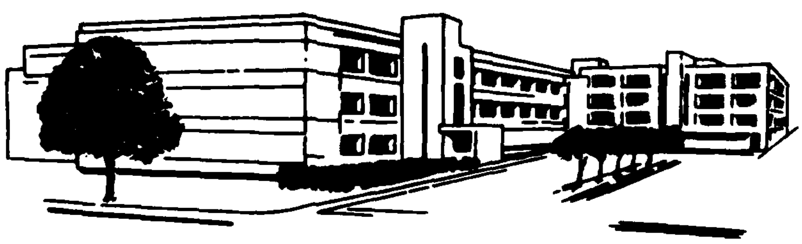
INSTITUTE REPORT NO. 110

A COMPUTER PROGRAM TO PROCESS SPECTROPHOTOMETRIC ANALYTICAL DATA ASSOCIATED WITH CURVILINEAR ABSORBANCE/CONCENTRATION RELATIONSHIPS.

JOHN J. KNUDSEN, BS
EVELYN L. McGOWN, PhD

ANALYTICAL CHEMISTRY GROUP
DIVISION OF RESEARCH SUPPORT

NOVEMBER 1981



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

INSTITUTE REPORT NO. 110

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A computer program, designed to provide a simple and accurate method for evaluating spectrophotometric assay data, accomodate both linear and nonlinear standard curves. The program requires manual entry by keyboard of absorbance and concentration values for the standard curve and absorbance values for the test samples. It will process up to 4 dilutions of each test sample and thus it can accommodate batches of samples containing widely differing concentrations of test compound. Aregression equation (up to 4th degree) is calculated to describe the standard

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curve with concentration as the independent variable and absorbance as the dependent variable. Concentrations in unknown samples are calculated from absorbances by using a method of successive approximations. A hard copy data printout lists input data, concentration of test substance in each sample, statistical data concerning the estimate of the standard curve, and a plot of the standard curve.

ABSTRACT

A computer program, designed to provide a simple and accurate method for evaluating spectrophotometric assay data, accommodate both linear and nonlinear standard curves. The program requires manual entry by keyboard of absorbance and concentration values for the standard curve and absorbance values for the test samples. It will process up to 4 dilutions of each test sample and thus it can accommodate batches of samples containing widely differing concentrations of test compound. A regression equation (up to 4th degree polynomial) is calculated to describe the standard curve with concentration as the independent variable and absorbance as the dependent variable. Concentrations in unknown samples are calculated from absorbance by using a method of successive approximations. A hard copy data printout lists input data, concentration of test substance in each sample, statistical data concerning the estimate of the standard curve, and a plot of the standard curve.

Preface

We acknowledge Dr. Jack Owicki; Department of Biophysics and Medical Physics; University of California, Berkeley, who wrote portions of an early version of the program. Two subroutines (BSECT and Monton) were modified and incorporated into the present program. We also thank Lottie Applewhite, LAIR Technical Editor, for her editoral assistance.

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Some spectrophotometric procedures in analytical chemistry do not conform to Beer's law (1). Examples of nonlinearity between concentration and absorbance include the thiocyanate assay for iron (2), the Lowry method for protein (3), and the method for chloride recommended by Technicon (4). Nonlinearity is especially characteristic of microbiological assays for nutrients (5).

Deviation from Beer's law may occur when the absorption band (or emission line) is not completely resolved (1). Most spectrophotometers employ a finite group of frequencies. The wider the bandwidth of radiation passed by the filter, the greater the apparent deviation from Beer' law. The deviation is generally more evident at higher concentrations and the curve bends toward the concentration (x) axis. Also discrepancies are also usually found when the absorbing solute dissociates or associates in solution, because the nature of the species in solution will vary with concentration. Scattered light from suspensions, fluorescence, or stray light may also cause deviation from Beer's law (1).

When an analyst arbitrarily draws a straight line or estimates a linear slope through standard curve points which are not linear, but curve toward the x axis, the result is underestimation of test samples with low concentrations and overestimation of the high samples. When the nonlinearity is severe enough, the analyst is forced to refer to the standard curve for each test sample. This is tedious and highly subject to human error. The program described in this report was created to eliminate the need for visual reference to a standard curve and to speed up the processing of data from such analyses. The program is also convenient for linear standard curves when several dilutions per test sample are assayed.

PROGRAM DESIGN

A computer program was written to perform the calculations necessary for analysis of curvilinear spectrophotometric absorbance/concentration data. The computer hardware and commercial software necessary for implementation of the program are presented under Materials. Details of the program itself, including a discussion of mathematical algorithms used, a definition of terms required for data entry, and limitations of the program, are addressed in Methods. A step-by-step presentation of the program as encountered by the user during execution is included under USER'S GUIDE.

Materials

To make the program available to all potential users at the

Institute, it was written for and implemented on the Institute's central minicomputer, a Data General Corporation Eclipse C330 (a general purpose minicomputer with 512,000 words (16 bits each) of main memory running under control of Data General Corporation's Advanced Operating System with full timesharing support). The program is written in Fortran V (Data General Corp., Version 6.02, a superset of Fortran IV and ANSI Fortran including elements of IBM Fortran IV and Univac Fortran V). For regression and statistical analysis of standard curve data, the program incorporates a computational subroutine (RLFOR) written in Fortran V by the International Mathematical and Statistical Libraries (IMSL), Houston, Texas (6). This subroutine is part of the statistical software package available to all users of the Institute's central computing facility. Users can gain access to the program at any of the timesharing terminals (CRT or printer) located throughout the Institute. Hard copy output data from the program is available to the user on a Dataproducts 2230 Line Printer located at the Institute's central computing facility.

Methods

The following paragraphs present the mathematical algorithms used in the program, limitations, and special terms required for the user to understand and use the program.

The mathematical model used to relate absorbance to concentration data for purpose of generating a regression equation from user input standard curve data is the simple polynomial

$$y = a_o + a_1x^1 + \cdots\cdots + a_nx^n$$

where, x = standard concentration (independent variable)
y = observed absorbance (dependent variable)
n = maximum degree of polynomial (discussed below)

This model allows for the calculation of a regression equation which can vary from a linear model (where $n = 1$) up to a polynomial of maximum degree n. The maximum degree of the regression equation allowed by the program has been arbitrarily limited to four. We have found that polynomial equations up to a maximum degree of four (a quartic equation) can be used to model even those assays which exhibit extreme nonlinearity (e.g., microbiologial nutrient assays).

The IMSL subroutine (6) included in the program performs the computations required for a standard univariate curvilinear regression analysis. Orthogonal polynomals are used by the subroutine to compute the regression polynomial (7,8). In addition to calculating the regression coefficients of the polynomial, the subroutine also provides the usual analysis of variance table information along with other statistical inferences for each model parameter. The fitted model returned by the subroutine is

$$y_i = a_o + a_1 x_i + a_2 x_i^2 + \cdots + a_p x_i^p + e_i$$

where: y_i = predicted absorbance for i^{th} data point (dependent variable)

x_i = standard concentration for i^{th} data point (independent variable)

p = maximum degree of polynomial (limited to four in this program)

a_{o-p} = computed regression coefficients

i = 1,2,....., N (number of data points)

e_i = population random error

The population random errors corresponding to e_i are assumed to be uncorrelated, to have means of zero, and have variances σ^2. During execution of the program the user has the choice of computing either a polynomial regression model or a linear regression model (in cases where it is known that the *relationship between* absorbance and concentration is linear under given assay conditions.)

Because of the possibility that the generated polynomial will not be monotonic over the input concentration range, a subroutine has been incorporated into the program to detect this condition. If the generated polynomial is not monotonic over the input concentration range, the program will abort and the user will be instructed to check data for accuracy or prepare a new standard curve if necessary.

In order to facilitate the computation of sample concentration data from the calculated regression model and also to allow the analyst a certain degree of latitude in preparing standard curves, the concepts of "standard volume" and "standard equivalent volume" have been used (see Appendix A, Table 1 for definitions). In preparing the standard curve for a given assay, it will be assumed that the analyst has prepared one solution of known concentration (representing the maximum concentration in the curve) and from this solution will prepare the standard curve. For purposes of illustration, a hypothetical example is given and the following assay parameters are assumed:

a. Total volume of standard (including diluent) = 2.0 ml

b. Number of points in standard curve = 11.

The analyst would prepare his standard curve in the following manner.

STANDARD VOLUME* (ml)	VOLUME OF DILUENT (ml)	SS TOTAL VOLUME (ml)	STANDARD EQUIVALENT VOLUME (ml)
(0.0)	2.0	2.0	0.0
(0.2)	1.8	2.0	0.1
(0.4)	1.6	2.0	0.2
(0.6)	1.4	2.0	0.3
(0.8)	1.2	2.0	0.4
(1.0)	1.0	2.0	0.5
(1.2)	0.8	2.0	0.6
(1.4)	0.6	2.0	0.7
(1.6)	0.4	2.0	0.8
(1.8)	0.2	2.0	0.9
(2.0)	0.0	2.0	1.0

The user enters only the "Standard Volume" (noted by an asterisk above) along with its corresponding absorbance during execution of the program. The "standard volume" is scaled by the program over the range: $0.0 \leq$ STANDARD EQUIVALENT VOLUME ≤ 1.0 for computational purposes within the program and for graphical presentation of the generated regression curve in the hardcopy data output of the program. Because of this scaling feature, the user is free to choose the assay volume to suit the test and/or instrumentation requirements. The maximum absorbance accepted by the program in its current form is 2.0. If transmittance values are determined instead of absorbance units, they must be converted by the user into absorbance units before the data can be entered in the program. A total of 30 standard curve data points (i.e., absorbance, standard volume pairs) can be input.

Upon successful completion of the regression equation (see Appendix A, Table 2 for error code descriptions), the program accepts certain alphameric information used in the hardcopy data output. This information consists of 1) name of assay, 2) type of sample, 3) date of assay, and 4) name of investigator. Complete instructions for entering this information are provided under USER'S GUIDE. Standard concentration and sample dilution factor information is also entered at this time, along with the number of dilutions per sample and the volume of the sample at each dilution. The program will accept up to 4 dilutions per sample. Finally, the sample number and the corresponding measured absorbance(s) (if more than one sample volume was prepared) for each sample are entered.

The mathematical technique used in this program to compute concentration from measured absorbance is a numerical iterative procedure commonly referred to as the method of successive approximations. Because the standard curve regression equation was generated with absorbance (dependent variable) as a function of concentration (independent variable), the equation must be either solved analytically for concentration as a function of absorbance or numerical techniques must be applied to arrive at a solution. Although analytical methods are available for the closed solution of equations up to the third degree, no generally applicable technique exists for the solution of quartic and higher degree equations (9,10). Iterative techniques approximate the root(s) of equations to some prescribed degree of accuracy. The subroutine employed in this program computes the unknown concentration to $\pm$ 1.0 x 10^{-5} standard equivalent volume. A complete description of this technique is presented in Appendix B, under Subroutine 'BSECT'.

After the unknown "standard equivalent volume" has been determined from the measured absorbance, the following relationship is used to compute

the actual concentration of substance in the sample(s).

[SUBSTANCE] = (STD.EQ.VOL)(SS.TOT.VOL)(STD.CONC.)(1/SD.VOL)(DF)

where:

SUBSTANCE = concentration (or amount/unit weight) of test substance in original sample.

STD.EQ.VOL. = Standard equivalent volume (calculated by BSECT subroutine).

SS.TOT.VOL. = sample and standard total volume.

STD.CONC. = concentration of standard used to prepare curve.

SD.VOL. = sample dilution volume.

DF = dilution factor.

(See Appendix A, Table 1 for further explanations of the abbreviations.)

The preceding calculation is based on SS total volume and is therefore independent of total reaction volume. For purposes of this program, the amounts of any additional reagents do not affect the calculations.

The program also provides the option of calculating more than one set of sample data (with different dilution factors, sample volumes, etc.) from the same standard curve regression equation. A complete listing of the program is provided in Appendix B.

USER'S GUIDE

General

Before giving a detailed description of the program from the user's point of view, two points must be discussed. The first deals with limitations of the program and the second is related to provisions made for 'unknown' sample data entry.

This program provides the analyst with a simple and accurate computational method for evaluating spectrophotometric assay data as an alternative to graphical techniques which are usually tedious and error-prone. The program generates a linear or polynomial regression equation from input standard curve data points. This equation is then used as a statistical model to estimate unknown sample concentration data from known absorbances. The program should not be used for purposes of inferring information about the nature of the chemical reaction involved in a particular chemical or microbiological assay (e.g. reaction mechanisms or kinetics).

Provision has been made in the program to allow entry of up to four dilutions per 'unknown' test sample. There are several reasons for including this feature. First, interfering substances can often be detected by assaying the test sample at several different dilutions. This problem is common in microbiological assays and is referred to as "upward" or "downward" drift, depending on whether the nonspecific interference is stimulatory or inhibitory. Thus for some assays, this feature could be considered as a monitor of the validity of the results for each sample. Secondly, with several dilutions, the analyst can handle samples with widely different concentrations of test substance without being forced to repeat samples which fall out of the range of the standard curve. It is often time-effective (particularly if automated pipetting equipment is available) to include extra dilutions and to disregard outlying values. Finally, the feature increases the statistical power of the resultant data by increasing the number of replicates.

Specific User Instructions

In the complete operational description of the program, capitalized sequences denote program output displayed on the user's terminal, underlined sequences denote prompts requiring user data input, and the appropriate user response is enclosed in parentheses. The program is outlined in the following nine steps.

STEP 1.) The user accesses the program at a timesharing terminal by entering the CLI command 'EXECUTE ASSAY'. A brief introduction to the program will be displayed on the terminal. The user presses <RETURN> to resume program execution.

STEP 2.) Instructions are given for entering standard curve data points, then the following series of prompts are displayed for data point entry.

DATA POINT NO.: 'n'

ENTER VOLUME OF STANDARD ('IND VARIABLE'):
(User enters volume of standard used for given data point.)

ENTER MEASURED ABSORBANCE FOR ABOVE VOLUME ('DEP' VARIABLE):
(User enters absorbance. Allowed absorbance range is 0.0 to 2.0.)

EXAMINE ABOVE ENTRIES. IF YOU WISH TO CORRECT

EITHER OR BOTH VALUE(S), ENTER '1' BELOW.

.....ENTER '1' TO CORRECT DATA POINT ENTRY.

.....ENTER '2' TO CONTINUE DATA POINT ENTRY.

.....ENTER '999' TO TERMINATE DATA POINT ENTRY.

ENTER CHOICE: (User enters '1' to correct data point entry just made, '2' to enter another data point, or '999' to terminate data point entry.)

At least five data points are recommended for linear standard curves and at least eight data points are recommended for nonlinear standard curves. A maximum of thirty data points may be entered.

STEP 3.) After all standard curve data points have been entered, the program outputs to the terminal the following message.

ENTER TYPE OF REGRESSION CURVE DESIRED -- LINEAR OR POLYNOMIAL

.....ENTER '1' FOR LINEAR REGRESSION CURVE.

.....ENTER '2' FOR POLYNOMIAL REGRESSION CURVE.

<u>ENTER CHOICE:</u> (User enters '1' if the assay is known to be linear, or '2' if a polynomial regression curve is desired.)

STEP 4.) Program displays the following message at the terminal:

CALCULATING POLYNOMIAL REGRESSION EQUATION.

If any error conditions are detected by the regression subroutine, a message indicating the fact will appear at the terminal. The most likely cause for an error to be detected is an insufficient number of data points for the requested type of regression equation. If an error occurs, the program must be aborted and the cause corrected before the user can continue. Possible error conditions are listed in Appendix A, Table 2.

If the regression equation was successfully computed, the following message appears at the terminal:

POLYMONIAL REGRESSION EQUATION OF DEGREE 'n' HAS BEEN CALCULATED.

STEP 5.) The user is next requested to enter information to annotate output data. The following prompts are displayed:

<u>ENTER TYPE OF ASSAY:</u>

User enters discription of assay. (Maximum, 32 alphameric characters.)

ENTER DATE OF ASSAY:

(User enters the date assay was conducted. Maximum of 32 alphameric characters may be entered.)

ENTER TYPE OF SAMPLE:

(User enters description of sample. Maximum of 32 alphameric characters may be entered.)

ENTER NAME OF INVESTIGATOR:

(User enters name of person conducting assay. Maximum of 32 alphameric characters may be entered.)

STEP 6.) Information required to calculate 'unknowns' is now entered. These data consist of dilution factor information, concentration of standard, the number of sample dilutions and the volume of each sample dilution.

STANDARD CONCENTRATION DATA:

.....ENTER NUMERICAL PORTION OF STANDARD CONCENTRATION:

(User enters numerical portion of 'standard' concentration.)

.....ENTER UNITS OF STANDARD:

(User enters units of standard (e.g., ng/ml, mg/ml, etc. A maximum of 6 characters may be entered.)

ENTER INITIAL DILUTION FACTOR OF SAMPLE MATERIAL:

(User enters initial dilution factor of sample material.)

If initial dilution factor is greater than 1000, the user will be requested to re-enter the units of the standard. For example, if the original units were 'ng/ml' then 'ug/ml' should be entered, or if the original units were 'mg/l' then g/l should be entered.

ENTER NUMBER OF SAMPLE DILUTIONS PER SAMPLE (MIN. 1, MAX. 4):

(User enters number of sample dilutions made for each sample. If only one, a '1' must be entered. Maximum that can be entered is '4'.)

ENTER 'MLS OF SAMPLE' AT DILUTION 'n':

(User enters the number of milliliters of sample used at each dilution volume.)

STEP 7.) Unknown absorbances and sample numbers are now entered into the program. A brief description of the sample entry procedure is displayed at the terminal, then the following prompts are given.

ENTER SAMPLE NUMBER:

(User enters a numerical sample number. The range the sample number can assume is from 1 to 99999.)

ENTER ABSORBANCE AT SAMPLE DILUTION 'n':

(User enters measured absorbance at indicated sample dilution.)

EXAMINE ABOVE DATA. IF YOU WISH TO CORRECT ANY

ENTRY, ALL DATA FOR SAMPLE (n) MUST BE RE-ENTERED.

.....ENTER '1' TO CORRECT DATA.

.....ENTER '2' TO CONTINUE WITH DATA ENTRY.

.....ENTER '999' TO TERMINATE DATA ENTRY.

ENTER CHOICE:

(User enters '1' if he wishes to correct a data entry just made, '2' to enter data for another sample, or '999' to terminate data entry.)

The user may enter absorbance data for up to fifty (50) samples.

STEP 8.) The user is now given the option of entering another set of data using the same regression curve. For example, if the user had data for more than 50 samples, he could use this feature to avoid the necessity of re-calculating the standard curve. This feature is also helpful if some samples had different initial treatment (e.g., different dilution factor).

DO YOU WISH TO ENTER MORE DATA USING SAME STANDARD CURVE?

.....ENTER '1' TO COMPUTE ANOTHER DATA SET.

.....ENTER '2' TO EXIT PROGRAM.

ENTER CHOICE:
(User enters '1' if he wishes to compute another set of data using same regression curve, or '2'to exit program.)

STEP 9.) At this point the following message is output to users terminal and program execution ceases.

OUTPUT DATA FILE 'ASSAY.DATA' CREATED. END OF PROGRAM.

An output data file named 'ASSAY.DATA' was created by the program in the user's directory. A hardcopy printout of this file can be obtained at the line printer located in the Institute's central computing facility by entering the CLI command 'QPRINT ASSAY.DATA'.

Hardcopy output provided by the program to the user consists of regression equation statistical data (analysis of variance table information along with statistical inferences pertaining to computed regression coefficients), input standard curve data points, and predicted values of absorbance (dependent variable) calculated using the regression model. A low resolution graph of both the input standard curve data points and the computed regression equation is provided for visual inspection of the curve. Sample data output includes assay parameters, input sample absorbances along with corresponding actual concentration of test substance in the sample material. If more than one dilution per test sample was performed, the mean concentration is calculated. The output includes not only the mean of all calculated values for each sample, but also an 'adjusted mean'. The choice of the range (mean $\pm$ 10%) for accepting values for the adjusted mean was purely arbitrary. The purpose of this feature is not only to eliminate spurious values, but also to allow the analyst to ascertain the variability of the assay. By scanning one column in the output, one can quickly determine how many (if any) dilutions yielded values which were more than 10% above or below the mean. Examples of data output for both a linear and nonlinear assay are given in Appendix C.

CONCLUSIONS

The computer program described in this report provides a simple and accurate method for evaluating spectrophotometric assay data. It is a general purpose program which will process any analytical data that must be referred to a standard curve. It will accommodate up to 4 dilutions per sample and thus can handle batches of analyses with widely differing concentrations of test substance. The program is available to anyone at this Institute.

RECOMMENDATIONS

None.

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TABLE 1 - DEFINITION OF TERMS USED IN PROGRAM

STANDARD VOLUME: Actual volume (in milliliters) of standard used to prepare each individual point in standard curve.

SS. TOTAL VOLUME: Standard or sample total volume (volume of standard or sample plus diluent). This value must equal maximum volume of standard.

STANDARD EQUIVALENT VOLUME: Input standard volumes scaled over the range of 0.0 to 1.0 inclusive. The standard volumes are scaled over this range for computational purposes and graphical presentation of the standard curve in the program output data.

DILUTION FACTOR: Initial dilution factor of sample material which occurs during sample preparation including dilution necessary to bring absorbance of test substance into range of the standard curve.

STANDARD CONCENTRATION: Concentration of test substance in the standard used to prepare the standard curve.

SAMPLE DILUTION VOLUME: Actual volume of sample (in milliliters) included in reaction mixture. This volume plus diluent must equal the SS.TOTAL VOLUME.

MEAN CONCENTRATION: Computed average concentration of test substance in sample if more than one sample dilution volume analyzed.

ADJUSTED MEAN CONCENTRATION: Computed average concentration of test substance in sample excluding those values exceeding ± 10% of the computed mean concentration if more than one sample dilution volume analyzed.

APPENDIX A

TABLE 2 - PROGRAM ERROR CODES

STANDARD CURVE ENTRY: All errors detected by the program which are related to standard curve data point entry are fatal to program execution. The user must correct the problem in the data and then access the program again. Errors at this point in the program are always caused by failure to enter at least three data points for a linear regression model, or a minimum of five data points for a polynomial regression model. If an error is detected, the following message will be displayed on the users terminal: STOP "INADEQUATE NUMBER OF DATA POINTS".

SAMPLE HARDCOPY DATA OUTPUT: The following error conditions may be detected by the program during computation of output data.

1.) If measured absorbance of test substance is outside the range of the computed standard curve regression model, an error code of '-111' will appear in the output data of the program in the appropriate column. To correct this error the user must dilute sample material to bring concentration of the test substance into range of the standard curve.

2.) if the concentration of test substance at all dilution volumes for a given sample exceeded $\pm$ 10% of the computed mean concentration, an error code of '-333' will appear in the 'ADJUSTED MEAN CONCENTRATION' column of the output data.

APPENDIX A

```
C ***********************************************************************
C *                                                                     *
C * ASSAY DATA ANALYSIS PROGRAM  -  PROGRAM NAME: "ASSAY"               *
C *                                                                     *
C * WRITTEN  -  MAY 1981  -  J.J. KNUDSEN, LAIR, DIV.OF RESEARCH        *
C *                          SUPPORT, ANALYTICAL CHEMISTRY GROUP        *
C *                                                                     *
C * LANGUAGE  -  DATA GENERAL FORTRAN V                                 *
C *                                                                     *
C * PURPOSE                                                             *
C * -------                                                             *
C * PROGRAM TO PROCESS SPECTROPHOTOMETRIC ANALYTICAL DATA ASSOCIATED    *
C * WITH CURVILINEAR ABSORBANCE/CONCENTRATION RELATIONSHIPS             *
C *                                                                     *
C * PROGRAM SYMBOL DEFINITION TABLE (EXCLUDING LOOP CONTROL INDEXES)    *
C * ----------------------------------------------------------------    *
C * INTEGER VARIABLES                                                   *
C * ------- ---------                                                   *
C * IX - ARGUMENT SUBRT 'RLFOR'.INPUT ROW DIM OF MATRIX 'XYW'           *
C * N - ARGUMENT SUBRT 'RLFOR'.INPUT NUMBER OF DATA POINTS (STD CURVE)  *
C * MDP - ARGUMENT SUBRT 'RLFOR'.CONTROL VECTOR OF LENGTH 3             *
C * IB - ARGUMENT SUBRT 'RLFOR'.INPUT ROW DIMENSION OF MATRIX 'B'       *
C * IP - ARGUMENT SUBRT 'RLFOR'.INPUT ROW DIMENSION OF MATRIX 'PRED'    *
C * IER - ARGUMENT SUBRT 'RLFOR'.ERROR PARAMETER                        *
C * IERROR - ARGUMENT SUBRT 'MONOT'.ERROR PARAMETER                     *
C * IERR - ARGUMENT SUBRT 'BSECT'.ERROR PARAMETER                       *
C * NVOL - MAX NUMBER OF DILUTION VOLUMES FOR GIVEN ASSAY               *
C * SNUM - INPUT VARIABLE. TEMPORARILY STORE SAMPLE NUMBER              *
C * SNUMBER - VECTOR OF MAX LENGTH 50 TO HOLD SAMPLE NUMBERS            *
C * GRAPH - TWO DIMENSIONAL MATRIX TO HOLD LOW RESOLUTION GRAPH         *
C * LABEL1 - VECTOR OF LENGTH 51 TO HOLD VERT AXIS LABEL OF 'GRAPH'     *
C * A - TEST INTEGER AND ARGUMENT OF SUBRT 'PLOT2'                      *
C * MTEST - TEST INTEGER.DETERMINES IF MORE THAN ONE SAMPLE DATA        *
C *         ANALYSIS WILL BE PERFORMED USING SAME 'STANDARD CURVE'      *
C * ITEST - TEST INTEGER.CONTROLS TYPE OF REGRESSION CURVE CALCULATED   *
C *                                                                     *
C * REAL VARIABLES                                                      *
C * ---- ---------                                                      *
C * ABSORBANCE - TWO DIM MATRIX TO HOLD INPUT SAMPLE ABSORBANCES        *
C * CONC - TWO DIM MATRIX TO HOLD DATA FOR SAMPLE 'UNKNOWN' CONC        *
C * LABEL2 - VECTOR OF LENGTH 51 TO HOLD VERT AXIS LABEL OF 'GRAPH'     *
C * MCPLUS - VARIABLE TO HOLD 'MEAN CONC + (MEAN CONC)*Q DATA           *
C * MCMINUS - VARIABLE TO HOLD 'MEAN CONC + (MEAN CONC)*Q DATA          *
C * STAND - VARIABLE TO HOLD NUMERICAL PORTION OF STANDARD CONC         *
C * DILFAC - VARIABLE TO HOLD SAMPLE DILUTION FACTOR                    *
C * SVOL - VARIABLE TO TEMPORARILY HOLD INPUT SAMPLE VOLUMES            *
C * SVOLUME - VECTOR OF LENGTH 4 TO HOLD INPUT SAMPLE VOLUMES           *
C * DAT - TWO DIM MATRIX TO HOLD STANDARD CURVE INPUT DATA              *
C * MAXVOL - VARIABLE TO HOLD MAXIMUM STANDARD CURVE INPUT VOLUME       *
C * MAXABS - VARIABLE TO HOLD MAXIMUM STANDARD CURVE INPUT ABSORBANCE   *
```

APPENDIX B

```
C * VALUE - VARIABLE TO TEMPORARILY HOLD INPUT STD CURVE INPUT DATA        *
C * DIV - VARIABLE TO DIVIDE SAMPLE CONC BY 1000 IF 'DILFAC' > 1000.       *
C * Q - VARIABLE SET TO .1 TO ARBITRARILY CONTROL EXCLUSION OF SAMPLE      *
C *     CONCENTRATION FROM MEAN ADJUSTED CONCENTRATION TABULATION          *
C *                                                                        *
C * DOUBLE PRECISION VARIABLES                                             *
C * ------ --------- ---------                                             *
C * XYW - ARGUMENT SUBRT 'RLFOR'.TWO DIM MATRIX (DATA POINT I/O)           *
C * RSQ - ARGUMENT SUBRT 'RLFOR'.CONTROL DEGREE OF FITTED MODEL            *
C * ALBP - ARGUMENT SUBRT 'RLFOR'.VECTOR OF LENGTH 2 CONTAINING RISK       *
C *        LEVELS                                                          *
C * ANOVA - ARGUMENT SUBRT 'RLFOR'.OUTPUT VECTOR OF LENGTH 13              *
C * B - ARGUMENT SUBRT 'RLFOR'.TWO DIM MATRIX(OUTPUT OF REGRESSION         *
C *     EQUATION COEFFICIENTS AND OTHER STATISTICAL DATA                   *
C * WK - ARGUMENT SUBRT 'RLFOR'.WORK VECTOR                                *
C * PRED - ARGUMENT SUBRT 'RLFOR'.OUTPUT 'N' BY 6 MATRIX                   *
C * XTOL - ARGUMENT SUBRT 'BSECT'. SET EQUAL TO TOLERANCE REQUIRED         *
C *        BY 'BSECT' SUBROUTINE                                           *
C * TEMP1 - TEMPORARY STORAGE VARIABLE                                     *
C * TEMP2 - TEMPORARY STORAGE VARIABLE                                     *
C * XLO - ARGUMENT SUBRTS 'BSECT','MONOT','PLOT2'.LOWER RANGE OF           *
C *       'STANDARD EQUIVALENT VOLUME'                                     *
C * XHI - ARGUMENT SUBRTS 'BSECT','MONOT','PLOT2'.UPPER RANGE OF           *
C *       'STANDARD EQUIVALENT VOLUME'                                     *
C * HD1 - VECTOR OF LENGTH 6 CONTAINING ALPHAMERIC ASSAY DATA              *
C * HD2 - VECTOR OF LENGTH 6 CONTAINING ALPHAMERIC ASSAY DATA              *
C * HD3 - VECTOR OF LENGTH 6 CONTAINING ALPHAMERIC ASSAY DATA              *
C * HD4 - VECTOR OF LENGTH 6 CONTAINING ALPHAMERIC ASSAY DATA              *
C * HD5 - VARIABLE CONTAINING ALPHAMERIC ASSAY DATA                        *
C * COEFF - VECTOR OF LENGTH 6 CONTAINING REGRESSION COEFFICIENTS          *
C * SABS - ARGUMENT SUBRT 'BSECT'.TRANSFER SAMPLE ABSORBANCE TO            *
C *        SUBRT 'BSECT'                                                   *
C *                                                                        *
C **************************************************************************


C PROGRAM DATA TYPE STATEMENTS

      INTEGER  IX,N,MDP(3),IB,IP,IER,IERROR,NVOL,SNUM,IERR,
     1         SNUMBER(50),GRAPH(0:50,0:100),LABEL1(51),A,
     1         ITEST,MTEST,NTEST

      REAL  ABSORBANCE(50,4),CONC(50,8),LABEL2(51),MCPLUS,MCMINUS,
     1      STAND,DILFAC,SVOL,SVOLUME(4),DAT(30,3),MAXVOL,MAXABS,
     1      VALUE,DIV,Q

      DOUBLE PRECISION  XYW(30,7),RSQ,ALBP(2),ANOVA(13),B(7,12),WK(100),
     1                  PRED(30,6),XTOL,TEMP1,TEMP2,XLO,XHI,HD6,
     1                  COEFF(6),HD1(4),HD2(4),HD3(4),HD4(4),HD5,SABS,X
```

```
C PROGRAM PARAMETERS

      PARAMETER  XHI=1.0, XLO=1.0E-8, XTOL=1.0E-5, Q=1.0E-1

C PROGRAM SYMBOL INITIALIZATION STATEMENTS

      DATA  (LABEL1(I),I=1,51)/' ',' ',' ',' ',' ',' ',' ',' ',' ',' ','
     1 ',' ',' ','S','T','A','N','D','A','R','D',' ','V','O','L','U','M'
     1,'E',' ','E','Q','U','I','V','A','L','E','N','T',' ',' ',' ',' ','
     1 ',' ',' ',' ',' ',' ',' ',' '/,(LABEL2(J),J=1,51)/'0.0',' ',' '
     1,' ',' ','0.1',' ',' ',' ',' ','0.2',' ',' ',' ',' ','0.3',' ',' '
     1,' ',' ','0.4',' ',' ',' ',' ','0.5',' ',' ',' ',' ','0.6',' ',' '
     1,' ',' ','0.7',' ',' ',' ',' ','0.8',' ',' ',' ',' ','0.9',' ',' '
     1,' ',' ','1.0'/

      RSQ=99.0
      MDP(3)=1
      ALBP(1)=0.05
      ALBP(2)=0.05
      IB=7
      IX=30
      IP=30
      N=0
      VALUE=0.0
      DIV=1.0
      A=0
      NTEST=0
      MTEST=0

C DELETE DATA PRINTOUT FILE IF FILE CURRENTLY IN EXISTANCE

      OPEN 1,"ASSAY.DATA",ATT="OP",LEN=130
      CLOSE 1
      DELETE "ASSAY.DATA"

C PROGRAM INTRODUCTION AND DESCRIPTION AS DISPLAYED ON TERMINAL

      TYPE"<NL><NL><NL>                    ASSAY DATA ANALYSIS PROGRAM"
      TYPE"<NL>PURPOSE:  FIT A LINEAR OR POLYNOMIAL REGRESSION EQUATION"
      TYPE"          TO A STANDARD CURVE USED TO CALIBRATE A CHEMICAL OR"
      TYPE"          MICROBIOLOGICAL ASSAY.  A MINIMUM OF FIVE DATA"
      TYPE"          POINTS ARE REQUIRED FOR A LINEAR REGRESSION ANALYSIS."
      TYPE"          A MINIMUM OF EIGHT DATA POINTS ARE RECOMMENDED FOR"
      TYPE"          A POLYNOMIAL REGREWWION ANALYSIS (MAXIMUM DEGREE OF"
      TYPE"          THE CALCULATED POLYNOMIAL IS 4).  AFTER REGRESSION"
      TYPE"          CURVE HAS BEEN CALCULATED, A MAXIMUM OF 50 UNKNOWNS"
      TYPE"          (ABSORBANCES) MAY BE ENTERED.  CONCENTRATION OF UN-"
      TYPE"          KNOWN CORRESPONDING  TO ENTERED ABSORBANCE WILL THEN"
      TYPE"          BE CALCULATED USING REGRESSION EQUATION.  FOLLOW"
      TYPE"          INSTRUCTIONS DISPLAYED ON TERMINAL FOR DATA ENTRY."
```

```
      TYPE"<NL><NL>"

      PAUSE "PRESS <RETURN> TO CONTINUE"

      TYPE"<NL><NL>STANDARD CURVE ENTRY ROUTINE:<NL>"

      TYPE"<NL>ENTER STANDARD CURVE DATA POINTS AT THIS TIME, IN"
      TYPE"INCREASING 'STANDARD VOLUME' ORDER.  WHEN LAST DATA POINT"
      TYPE"HAS BEEN ENTERED, IT IS NECESSARY TO ENTER '999' TO TERMINATE"
      TYPE"STANDARD CURVE ENTRY ROUTINE.<NL>"

C STANDARD CURVE DATA POINT ENTRY ROUTINE

20       N=N+1
           TYPE"--------------------------------------<NL>"
24         WRITE(10,1000) N
           WRITE(10,1005)
           READ FREE (11) VALUE
           DAT(N,1)=VALUE
           WRITE(10,1010)
           READ FREE (11) VALUE
           DAT(N,2)=VALUE

           TYPE"<NL>EXAMINE ABOVE ENTRIES.  IF YOU WISH TO CORRECT"
           TYPE"EITHER OR BOTH VALUE(S), ENTER '1' BELOW.<NL>"

26         TYPE".....ENTER '1' TO CORRECT DATA POINT ENTRY"
           TYPE".....ENTER '2' TO CONTINUE WITH DATA POINT ENTRY"
           TYPE".....ENTER '999' TO TERMINATE DATA POINT ENTRY"

           ACCEPT"<NL>ENTER CHOICE: ", I

           IF(I.EQ.1) GO TO 24
           IF(I.EQ.2) GO TO 28
           IF(I.EQ.999) GO TO 30
             GO TO 26

28       GO TO 20

30     TYPE"<NL><NL><NL><NL>"
       TYPE"ENTER TYPE OF REGRESSION CURVE DESIRED -- LINEAR OR POLYNOMIA
      1L<NL>"

       TYPE".....ENTER '1' FOR LINEAR REGRESSION CURVE."
       TYPE".....ENTER '2' FOR POLYNOMIAL REGRESSION CURVE."

       ACCEPT"<NL>ENTER CHOICE: ", ITEST

          IF(ITEST.EQ.1) MDP(1)=1
          IF(ITEST.EQ.2) MDP(1)=4
```

```
      IF(ITEST.NE.1.AND.ITEST.NE.2) GO TO 30

C DETERMINE MAXIMUM VOLUME (MAXVOL) AND MAXIMUM ABSORBANCE
C (MAXABS) OF STANDARD CURVE.

34    MAXABS=0.
      MAXVOL=0.
      DO 50 I=1,N-1
        IF(DAT(I+1,1).GT.DAT(I,1)) MAXVOL=DAT(I+1,1)
        IF(DAT(I+1,2).GT.DAT(I,2)) MAXABS=DAT(I+1,2)
50    CONTINUE

C SET A=1 IF 'MAXABS' > 1.0

      IF(MAXABS.GT.1.) A=1

C DIVIDE INPUT STANDARD VOLUME BY 'MAXVOL' TO SCALE RANGE OF
C STANDARD CURVE OVER INTERVAL 0.00 TO 1.00.  TRANSFER STANDARD
C CURVE FROM ARRAY 'DAT' TO ARRAY 'XYW' FOR INPUT TO SUBROUTINE
C 'RLFOR'.  SET 'XYW(I,3)' = 1.0 FOR UNWEIGHTED REGRESSION COEFFICIENTS.

      DO 60 I=1,N
        DAT(I,3)=DAT(I,1)/MAXVOL
        XYW(I,1)=DAT(I,3)
        XYW(I,2)=DAT(I,2)
        XYW(I,3)=1.0
60    CONTINUE

C CALCULATE REGRESSION EQUATION USING IMSL SUBROUTINE  'RLFOR'.

70    TYPE"<NL><NL>CALCULATING POLYNOMIAL REGRESSION EQUATION.<NL><NL>"

      CALL RLFOR (XYW,IX,N,RSQ,MDP,ALBP,ANOVA,B,IB,PRED,IP,WK,IER)

C ABORT PROGRAM IF 'IER' GREATER THAN 100

      IF(IER.GE.100) STOP "INADEQUATE NUMBER OF DATA POINTS"

C TRANSFER REGRESSION EQUATION COEFFICIENTS TO ARRAY 'COEFF'.

      COEFF(1)=B(MDP(2)+1,2)
      DO 80 I=1,MDP(2)
        COEFF(I+1)=B(I,2)
80    CONTINUE

C IF LINEAR REGRESSION EQUATION CALCULATED DO NOT DETERMINE
C MONOTINICITY OF EQUATION.

      WRITE(10,1210) MDP(2)
```

```
85      IF(ITEST.EQ.1) GO TO 100

C IF POLYNOMIAL REGRESSION EQUATION CALCULATED USE SUBROUTINE
C 'MONOT' TO DETERMINE IF EQUATION IS MONOTONIC.

        CALL MONOT (COEFF,MDP(2),XHI,XLO,IERROR)

C IF REGRESSION EQUATION NOT MONOTONIC NOTIFY USER TO RECHECK
C STANDARD CURVE DATA FOR CORRECTNESS OR SUGGEST HE PREPARE NEW
C STANDARD CURVE.

        IF(IERROR.EQ.0) GO TO 100
          NTEST=1

      TYPE"<NL><NL><NL>CAUTION: CALCULATED REGRESSION EQUATION IS NOT"
      TYPE"MONOTONIC. SUGGEST USER RE-CHECK STANDARD CURVE DATA FOR"
      TYPE"ACCURACY.  IF STANDARD CURVE DATA ENTERED CORRECTLY USER"
      TYPE"SHOULD CONSIDER PREPARING NEW STANDARD CURVE.  UNKNOWN"
      TYPE"SAMPLE DATA WILL NOT BE ACCEPTED BY THE PROGRAM. STATISTICAL"
      TYPE"DATA AND PLOT OF REGRESSION EQUATION IS AVAILABLE TO USER"
      TYPE"IN FILE NAMED 'ASSAY.DATA'. <NL><NL>"


      PAUSE   "PRESS <RETURN> TO CONTINUE"

        GO TO 302

C INPUT AT TERMINAL ASSAY INFORMATION (NAME, DATE OF ASSAY,TYPE,
C INVESTIGATOR AND INFORMATION REQUIRED FOR UNKNOWN CONCENTRATION
C CALCULATION.

100     WRITE(10,1230)
        READ(11,1250) (HD1(I),I=1,4)
        WRITE(10,1235)
        READ(11,1250) (HD2(I),I=1,4)
        WRITE(10,1240)
        READ(11,1250) (HD3(I),I=1,4)
        WRITE(10,1245)
        READ(11,1250) (HD4(I),I=1,4)
        WRITE(10,1255)
        WRITE(10,1260)
        READ FREE (11) STAND
        WRITE(10,1265)
        READ(11,1270) HD5
        WRITE(10,1275)
        READ FREE (11) DILFAC

        IF(DILFAC.GE.1000.) GO TO 130
          GO TO 135
```

```
130     WRITE(10,1276)
        WRITE(10,1277)
        READ(11,1270) HD6
        DIV=1000.

135     WRITE(10,1280)
        READ(11,1285) NVOL

        IF(NVOL.LT.1.OR.NVOL.GT.4) GO TO 135

        DO 140 I=1,NVOL
          WRITE(10,1290) I
          READ FREE (11) SVOL
          SVOLUME(I)=SVOL
140     CONTINUE

C INITIALIZE ELEMENTS OF 'CONC' ARRAY TO ZERO.

        DO 145 I=1,50
          DO 145 J=1,8
            CONC(I,J)=0.
145     CONTINUE

     TYPE"<NL><NL>SAMPLE DATA ENTRY ROUTINE<NL>"
     TYPE"      1.) ON PROMPT - ENTER SAMPLE NUMBER."
     TYPE"      2.) ON PROMPT - ENTER 'ABSORBANCE' AT INDICATED DILUTION"
     TYPE"                      VOLUME."
     TYPE"      3.) IF YOU ARE MISSING AN 'ABSORBANCE' AT INDICATED"
     TYPE"          DILUTION VOLUME, ENTER '0'.<NL><NL>"

        KT=0
150     KT=KT+1
        TYPE"----------------------------------------<NL>"
155     WRITE(10,1295) KT
        WRITE(10,1300)
        READ FREE (11) SNUM
        SNUMBER(KT)=SNUM

        DO 180 I=1,NVOL
          WRITE(10,1305) I
          READ FREE (11) SABS
          ABSORBANCE(KT,I)=SABS
          IF(ABSORBANCE(KT,I).GT.0.) GO TO 160
            CONC(KT,I)=0.0
            GO TO 180

160     CONTINUE

C USE SUBROUTINE 'BSECT' TO CALCULATE 'STANDARD EQUIVALENT VOLUME'
```

```
C FROM 'UNKNOWN' SAMPLE ABSORBANCE.

        CALL BSECT (SABS,X,COEFF,MDP(2),XLO,XHI,XTOL,IERR)

C IF IERR=0, SET CONC(KT,I)=X.  IF IERR=1, SET CONC(KT,I)=-111.
C IF IERR=2, SET CONC(KT,I)=-222.

        IF(IERR.EQ.0) CONC(KT,I)=X
        IF(IERR.EQ.1) CONC(KT,I)=-111.
        IF(IERR.EQ.2) CONC(KT,I)=-222.

180     CONTINUE

        IF(KT.EQ.50) GO TO 184
          GO TO 186

184     TYPE"<NL><NL>CAUTION: YOU HAVE ENTERED ABSORBANCE DATA FOR 50"
        TYPE"SAMPLES. YOU MUST NOW ENTER '999' BELOW AND TERMINATE DATA"
        TYPE"ENTRY. TO ENTER MORE ABSORBANCE DATA USING SAME STANDARD"
        TYPE"CURVE USE PROGRAM CONTINUATION OPTION TO BE PRESENTED"
        TYPE"BELOW.<NL><NL>"

186     TYPE"<NL>EXAMINE ABOVE DATA.  IF YOU WISH TO CORRECT"
        TYPE"ANY ENTRY, ALL DATA FOR SAMPLE ",SNUM," MUST"
        TYPE"BE RE-ENTERED.<NL>"
        TYPE".....ENTER '1' TO CORRECT DATA ENTRY."
        TYPE".....ENTER '2' TO CONTINUE WITH DATA ENTRY."
        TYPE".....ENTER '999' TO TERMINATE DATA ENTRY."

190     ACCEPT"<NL>ENTER CHOICE: ", I

        IF(I.EQ.1) GO TO 155
        IF(I.EQ.2) GO TO 150
        IF(I.EQ.999) GO TO 205
          GO TO 190

C CALCULATE SAMPLE 'UNKNOWN' CONCENTRATION FROM 'STANDARD VOLUME
C EQUIVALENT' OF 'UNKNOWN' OBTAINED FROM 'BSECT' SUBROUTINE.

205     DO 300 I=1,KT
          TEMP1=0.
          K=0
          DO 240 J=1,NVOL
            IF(CONC(I,J).GT.0.) GO TO  210
              GO TO 240
210         CONC(I,J)=(CONC(I,J)*MAXVOL*STAND*(1./SVOLUME(J))*DILFAC)
            CONC(I,J)=CONC(I,J)/DIV
            TEMP1=TEMP1+CONC(I,J)
            K=K+1
240       CONTINUE
```

```
          CONC(I,5)=TEMP1/K

C CALCULATE +/-(MEAN CONCENTRATION)*Q.  IF ANY INDIVIDUAL 'UNKNOWN'
C CONCENTRATION EXCEEDS +/-(MEAN CONC + (MEAN CONC)*Q) DO NOT INCLUDE
C THAT 'UNKNOWN' CONCENTRATION IN 'AVERAGE MEAN ADJUSTED CONCENTRATION'.

          MCPLUS=CONC(I,5)+(CONC(I,5)*Q)
          MCMINUS=CONC(I,5)-(CONC(I,5)*Q)

          TEMP2=0.
          K=0
          DO 270 L=1,NVOL
            IF(CONC(I,L).GT.0) GO TO 260
              GO TO 270
260         IF((CONC(I,L).GT.MCPLUS)     .OR.
     1        (CONC(I,L).LT.MCMINUS)       ) GO TO 270
              TEMP2=TEMP2+CONC(I,L)
              K=K+1

270         CONTINUE

            M=NVOL-K
            IF(K.EQ.0) GO TO 275
              GO TO 280
275         CONC(I,7)=-333.
            GO TO 290
280         CONC(I,7)=TEMP2/K
290         CONC(I,6)=M
            CONC(I,8)=K

300       CONTINUE

C TEST TO DETERMINE IF 'MTEST' FLAG HAS BEEN CHANGED TO '2'.  IF
C NOT, OPEN FLIE FOR INITIAL DATA ENTRY.  IF 'MTEST'= 2, BRANCH TO
C LINE 450 TO ADD NEW OUTPUT DATA TO FILE.

          IF(MTEST.EQ.1) GO TO 450

C OPEN CHANNEL '1' FOR USE IN CREATING DATA OUTPUT FILE.

302       OPEN 1,"ASSAY.DATA",ATT="OP",LEN=130

C CREATE FILE OF OUTPUT DATA NAMED 'ASSAY.DATA'.

          WRITE(1,1015)
          WRITE(1,1018)
          WRITE(1,1020) MDP(1)
          WRITE(1,1022) MDP(2)
          WRITE(1,1025)
```

```
      WRITE(1,1030)
      WRITE(1,1033)
      WRITE(1,1035)
      WRITE(1,1038)
      WRITE(1,1040) ANOVA(1),ANOVA(4),ANOVA(7),ANOVA(9),ANOVA(10)
      WRITE(1,1043) ANOVA(2),ANOVA(5),ANOVA(8)
      WRITE(1,1045) ANOVA(3),ANOVA(6)
      WRITE(1,1030)
      WRITE(1,1047)
      WRITE(1,1050)
      WRITE(1,1053)
      WRITE(1,1055)
      WRITE(1,1057)
      WRITE(1,1058)

      DO 400 J=1,MDP(2)
        WRITE(1,1060) J,(B(J,I),I=1,8)
400   CONTINUE

      WRITE(1,1063) (B(MDP(2)+1,I),I=1,8)

      WRITE(1,1050)
      WRITE(1,1065) ANOVA(11)
      WRITE(1,1067) ANOVA(12)
      WRITE(1,1070) IER
      WRITE(1,1085)
      WRITE(1,1090)
      WRITE(1,1093)
      WRITE(1,1030)
      WRITE(1,1095)
      WRITE(1,1097)
      WRITE(1,1098)
      WRITE(1,1100)
      WRITE(1,1103)

      DO 420 I=1,N
        RES=DAT(I,2)-PRED(I,1)
        WRITE(1,1105) I,DAT(I,1),DAT(I,3),DAT(I,2),PRED(I,1),RES
420   CONTINUE

      WRITE(1,1030)
      WRITE(1,1107)
      WRITE(1,1110)

C CALL PLOT1 AND PLOT2 SUBROUTINES TO CREATE LOW RESOLUTION GRAPH
C OF INPUT STD CURVE DATA POINTS AND CALCULATED REGRESSION EQUATION.

      CALL PLOT1 (GRAPH)
      CALL PLOT2 (GRAPH,N,DAT,MDP(2),COEFF,A)
```

```
      WRITE(1,1085)
      WRITE(1,1430)
      WRITE(1,1432)
      WRITE(1,1434)

      J=50
      I=1

430   WRITE(1,1435) LABEL1(I),LABEL2(I),(GRAPH(J,K),K=0,100)
        J=J-1
        I=I+1
        IF(J.GE.0) GO TO 430
      CONTINUE

      IF(A.EQ.0) GO TO 435
        GO TO 440
435   WRITE(1,1440)
        GO TO 445
440   WRITE(1,1442)
445   WRITE(1,1445)

C IF REGRESSION EQUATION NOT MONOTONIC DO NOT ADD UNKNOWN CONCENTRATION
C DATA TO OUTPUT DATA FILE.

      IF(NTEST.EQ.1) GO TO 360

450   WRITE(1,1310)
      WRITE(1,1315)
      WRITE(1,1320) (HD1(I),I=1,4),STAND,HD5
      WRITE(1,1325) (HD2(I),I=1,4),DILFAC
      WRITE(1,1330) (HD3(I),I=1,4),NVOL

      IF(DILFAC.GT.1000.) GO TO 304
        GO TO 306
304   WRITE(1,1335) (HD4(I),I=1,4),HD6
        GO TO 308
306   WRITE(1,1335) (HD4(I),I=1,4),HD5

308   DO 310 I=1,NVOL
        WRITE(1,1340) I,SVOLUME(I)
310   CONTINUE

      WRITE(1,1345)
      WRITE(1,1350)
      WRITE(1,1355)
      WRITE(1,1360)
      WRITE(1,1365)
      WRITE(1,1370) O
      WRITE(1,1375)
```

```
          DO 340 I=1,KT
            WRITE(1,1380) SNUMBER(I),(ABSORBANCE(I,J),J=1,4),
     1                    (CONC(I,K),K=1,8)
340       CONTINUE

          WRITE(1,1350)
          WRITE(1,1385)
          WRITE(1,1390)
          WRITE(1,1395)
          WRITE(1,1400)
          WRITE(1,1415)
          WRITE(1,1420) 0

350       TYPE"<NL><NL><NL><NL>"
          TYPE"DO YOW WISH TO ENTER MORE DATA USING SAME STANDARD CURVE?<
     1NL>"
          TYPE".....ENTER '1' TO CONTINUE DATA ENTRY"
          TYPE".....ENTER '2' TO EXIT PROGRAM"

          ACCEPT"<NL>ENTER CHOICE: ", MTEST
          TYPE"<NL><NL><NL><NL>"

          IF(MTEST.EQ.1) GO TO 100
          IF(MTEST.EQ.2) GO TO 360
          IF(MTEST.NE.1.AND.MTEST.NE.2) GO TO 350

360       CLOSE 1

C TERMINAL DATA INPUT AND STATISTICAL OUTPUT FORMAT STATEMENTS.

1000  FORMAT(/,"DATA POINT NO.:",I3,/)
1005  FORMAT(Z,"ENTER VOLUME OF STANDARD ('IND' VARIABLE): ")
1010  FORMAT(Z,"ENTER MEASURED ABSORBANCE FOR ABOVE VOLUME('DEP' VARIABL
     C1E): ")
1015  FORMAT(1X,"*** STATISTICAL DATA --> CALCULATED POLYNOMIAL REGRESSI
     1ON EQUATION ***")
1018  FORMAT(1X,"-----------------------------------------------------
     1---------------",//)
1020  FORMAT(1X,"REQUESTED DEGREE OF REGRESSION EQUATION:  ",I1)
1022  FORMAT(1X,"DEGREE OF CALCULATED REGRESSION EQUATION:  ",I1,/)
1025  FORMAT(1X,"** ANALYSIS OF VARIANCE **")
1030  FORMAT(1X,"-----------------------------------------------------
     1-----------------------------")
1033  FORMAT(1X,"*",2X,"SOURCE OF",8X,"DEGREES OF",3X,"SUMS OF",5X,"MEAN
     1",6X,"COMPUTED",6X,"TAIL AREA",6X,"*")
1035  FORMAT(1X,"*",2X,"VARIATION",9X,"FREEDOM",5X,"SQUARES",4X,"SQUARES
     1",4X,"F-VALUE",5X,"F-DISTRIBUTION",3X,"*")
1038  FORMAT(1X,3X,"---------",8X,"----------",3X,"-------",4X,"-------"
     1,4X,"-------",5X,"--------------",/)
1040  FORMAT(1X,3X,"REGRESSION",9X,F4.0,5X,F9.5,2X,F9.5,3X,F9.3,4X,F14.9
```

```
     1)
1043  FORMAT(1X,3X,"RESIDUAL",11X,F4.0,5X,F9.5,2X,F9.5)
1045  FORMAT(1X,3X,"CORRECTED TOTAL",4X,F4.0,5X,F9.5)
1047  FORMAT(//////,1X,"** REGRESSION COEFFICIENTS **")
1050  FORMAT(1X,"-----------------------------------------------------
     1---------------------------------------------------")
1053  FORMAT(1X,"*",50X,"LOWER",7X,"UPPER",6X,"ADJUSTED",3X,"PARTIAL",17
     1X,"*")
1055  FORMAT(1X,"*","DEGREE",5X,"VARIABLE",4X,"REGRESSION",4X,"STANDARD"
     1,3X,"CONFIDENCE",2X,"CONFIDENCE",3X,"SUMS OF",4X,"F-TEST",5X,"TAIL
     1 AREA",4X,"*")
1057  FORMAT(1X,"*","OF TERM",6X,"MEANS",4X,"COEFFICIENTS",4X,"ERROR",7X
     1,"LIMIT",7X,"LIMIT",6X,"SQUARES",4X,"VALUES",3X,"F-DISTRIBUTION",1
     1X,"*")
1058  FORMAT(2X,"-------",4X,"--------",3X,"------------",3X,"--------",
     13X,"----------",2X,"----------",3X,"--------",3X,"-------",2X,"---
     1----------",/)
1060  FORMAT(1X,1X,"X**",I1,7X,F8.5,2X,F13.8,3X,F8.5,3X,F8.4,4X,F8.4,4X,
     1F9.5,2X,F8.4,2X,F14.9)
1063  FORMAT(1X,1X,"INTERCEPT:",1X,F8.5,2X,F13.8,3X,F8.5,3X,F8.4,4X,F8.4
     1,4X,F9.5,2X,F8.4,2X,F14.9)
1065  FORMAT(//,1X,"PERCENTAGE OF VARIATION EXPLAINED BY ESTIMATED MODEL
     1: ",F10.6)
1067  FORMAT(1X,"STANDARD DEVIATION OF RESIDUALS: ",F10.6,/)
1070  FORMAT(1X,"POLYNOMIAL CALCULATION SUBROUTINE ERROR PARAMETER: ",I1
     1)
1085  FORMAT(1H1)
1090  FORMAT(1X,"*** INPUT DATA AND PREDICTED VALUES FROM MODEL ***")
1093  FORMAT(1X,"----------------------------------------------------",///
     1)
1095  FORMAT(1X,"*",17X,"-X-",9X,"-X-",51X,"*")
1097  FORMAT(1X,"*",16X,"INPUT",7X,"SCALED",9X,"-Y-",11X,"-Y-",23X,"*")
1098  FORMAT(1X,"*",2X,"DATA POINT",3X,"STANDARD",4X,"STANDARD",7X,"INPU
     1T",7X,"PREDICTED",20X,"*")
1100  FORMAT(1X,"*",4X,"NUMBER",6X,"VOLUME",4X,"EQ. VOLUME",4X,"ABSORBAN
     1CE",4X,"ABSORBANCE",4X,"RESIDUAL VALUE",1X,"*")
1103  FORMAT(1X,3X,"----------",3X,"--------",3X,"----------",4X,"------
     1----",4X,"----------",4X,"--------------",/)
1105  FORMAT(1X,7X,I2,7X,F7.4,5X,F7.4,8X,F6.4,8X,F6.4,9X,F7.5)
1107  FORMAT(/,1X,7X,"X  -->  INDEPENDENT VARIABLE")
1110  FORMAT(1X,7X,"Y  -->  DEPENDENT VARIABLE")
1210  FORMAT("POLYNOMIAL REGRESSION EQUATION OF DEGREE ",I1," HAS BEEN C
     1ALCULATED.",//)

C FORMAT STATEMENTS FOR UNKNOWN SAMPLE DATA OUTPUT AND INPUT.

1230  FORMAT(Z,"ENTER TYPE OF ASSAY: ")
1235  FORMAT(Z,"ENTER DATE OF ASSAY: ")
1240  FORMAT(Z,"ENTER TYPE OF SAMPLE: ")
1245  FORMAT(Z,"ENTER NAME OF INVESTIGATOR: ")
```

```
1250  FORMAT(4A8)
1255  FORMAT(/,"STANDARD CONCENTRATION DATA: ")
1260  FORMAT(".....ENTER NUMERICAL PORTION OF STANDARD CONCENTRATION: ",
     1Z)
1265  FORMAT(".....ENTER UNITS OF STANDARD (EXAMPLE: GM/ML): ",Z)
1270  FORMAT(A6)
1275  FORMAT(/,"ENTER INITIAL DILUTION FACTOR OF SAMPLE MATERIAL: ",Z)
1276  FORMAT(/,"CAUTION: DILUTION FACTOR GREATER THAN 1000",/,9X,"YOU MU
     1ST INCREASE UNITS OF STANDARD BY A FACTOR OF 1000.",//,9X,"IF UNIT
     1S OF STANDARD WERE 'NG/ML', CHANGE TO 'UG/ML' ",/,9X,"IF UNITS WER
     1E 'UG/ML', CHANGE TO 'MG/ML' ",/,9X,"IF UNITS WERE 'MG/ML', CHANGE
     1 TO 'GM/ML' ",/)
1277  FORMAT(Z,".....ENTER NEW UNITS FOR STANDARD: ")
1280  FORMAT(/,"ENTER NUMBER OF SAMPLE DILUTIONS PER SAMPLE (MINIMUM 1,
     1 MAXIMUM 4): ",Z)
1285  FORMAT(I1)
1290  FORMAT(".....ENTER 'MLS OF SAMPLE' AT DILUTION ",I1," : ",Z)
1295  FORMAT(/,"DATA ENTRY: ",I3,/)
1300  FORMAT("ENTER SAMPLE NUMBER: ",Z)
1305  FORMAT(".....ENTER ABSORBANCE AT SAMPLE DILUTION ",I1,": ",Z)
1310  FORMAT(1H1,"***ASSAY REGRESSION PROGRAM - SAMPLE DATA OUTPUT***")
1315  FORMAT(1X,"-----------------------------------------------------",/
     1/)
1320  FORMAT(1X,"TYPE OF ASSAY:",8X,4A8,9X,"ASSAY PARAMETERS -> STANDARD
     1 CONCENTRATION:",1X,F7.2,1X,A6)
1325  FORMAT(1X,"DATE OF ASSAY:",8X,4A8,9X,"----- ----------    SAMPLE D
     1ILUTION FACTOR:",1X,F7.2)
1330  FORMAT(1X,"SAMPLE TYPE:",10X,4A8,29X,"NO. OF SAMPLE DILUTIONS:",1X
     1,I1)
1335  FORMAT(1X,"NAME OF INVESTIGATOR:",1X,4A8,29X,"SAMPLE CONCENTRATION
     1 UNITS: ",A6,/)
1340  FORMAT(1X,83X,"SAMPLE DILUTION ",I1," = ",F5.3," ML ")
1345  FORMAT(/)
1350  FORMAT(1X,"-------------------------------------------------------
     1-------------------------------------------------------
     1-")
1355  FORMAT(1X,"*",11X,"*",31X,"*",31X,"*",8X,"*",5X,"ADJUSTED CONCENTR
     1ATION",8X,"*")
1360  FORMAT(1X,"*",11X,"*",6X,"A B S O R B A N C E",6X,"*",3X,"C O N C
     1 E N T R A T I O N",3X,"*",2X,"MEAN",2X,"*",2X,"NO.OF DIL VOLS",2X
     1,"*",1X,"ADJUSTED",1X,"*",1X,"NO.",1X,"*")
1365  FORMAT(1X,"*",1X,"SAMPLE NO.*",31X,"*",31X,"*",2X,"CONC",2X,"*",4X
     1,"EXCEEDING",5X,"*",3X,"MEAN",3X,"*",1X,"IN",2X,"*")
1370  FORMAT(1X,"*",11X,"*",1X,"DIL 1   DIL 2   DIL 3   DIL 4",1X,"*",1X
     1,"DIL 1   DIL 2   DIL 3   DIL 4",1X,"*",2X,"(MC)",2X,"*",1X,"+/-("
     1,F3.2,"*MC + MC)",1X,"*",3X,"CONC",3X,"*",1X,"AVG.","*")
1375  FORMAT(1X,1X,"----------  -----  -----  -----  -----  -----
     1 -----  -----  -----  ----  ---------------  -------  ---
     1--",/)
1380  FORMAT(1X,3X,I4,7X,F5.3,3X,F5.3,3X,F5.3,3X,F5.3,2X,F6.1,2X,F6.1,2X
```

```
     1,F6.1,2X,F6.1,3X,F6.1,1X,"[",8X,F2.0,11X,F6.1,4X,F2.0,2X,"]")
1385  FORMAT(//)
1390  FORMAT(1X,"ERROR CODES - CONCENTRATION CALCULATION ROUTINE")
1395  FORMAT(1X,"----------------------------------------------",/)
1400  FORMAT(1X,"      1.) -111. IN CONCENTRATION COLUMNS INDICATES THAT
     1 SAMPLE ABSORBANCES NOT IN RANGE OF STANDARD CURVE.",/)
1415  FORMAT(1X,"      2.) -333. IN ADJUSTED CONCENTRATION COLUMNS INDICA
     1TES THAT THE CONCENTRATION OF UNKNOWN AT ALL DILUTIONS")
1420  FORMAT(1X,"            EXCEEDED -> +/-(",F3.2,"*MC + MC).")
1430  FORMAT(1X,18X,"*** CALCULATED REGRESSION EQUATION - LOW RESOLUTION
     1 PLOT - [X-AXIS VERTICAL; Y-AXIS HORIZONTAL] ***",/)
1432  FORMAT(1X,82X,"SYMBOL FOR INPUT DATA POINTS:    -> 0")
1434  FORMAT(1X,82X,"SYMBOL FOR REGRESSION EQUATION: -> @",/)
1435  FORMAT(1X,10X,A1,2X,A3,1X,101A1)
1440  FORMAT(1X,16X,"0.0        0.1        0.2        0.3        0.4        0
     1.5        0.6        0.7        0.8        0.9        1.0",/)
1442  FORMAT(1X,16X,"0.0        0.2        0.4        0.6        0.8        1
     1.0        1.2        1.4        1.6        1.8        2.0",/)
1445  FORMAT(1X,57X,"A B S O R B A N C E",/)


9000  STOP "OUTPUT DATA FILE 'ASSAY.DATA' CREATED.  END OF PROGRAM"
9999  END
```

```
C ***********************************************************************
C *                                                                     *
C * SUBROUTINE 'BSECT'  -  CALLING PROGRAM NAME: 'ASSAY'               *
C *                                                                     *
C * WRITTEN MAY 1981  -  J.J.KNUDSEN, LAIR, DIV. OF RESEARCH SUPPORT    *
C *                      ANALYTICAL CHEMISTRY GROUP.                    *
C *                                                                     *
C * LANGUAGE  -  DATA GENERAL FORTRAN V                                 *
C *                                                                     *
C * PURPOSE OF SUBROUTINE                                               *
C * ------- -- ----------                                               *
C * POLY(X) IS A POLYNOMIAL IN X OF DEGREE 'NDEG'.                      *
C *                                                                     *
C *  POLY(X) = COEFF('ZERO') + SUM COEFF(I)*X**I                        *
C *                                                                     *
C *   WHERE: I = 1 TO NDEG                                              *
C *                                                                     *
C * POLY(X) IS DEFINED FOR 'X' BETWEEN 'XLO' AND 'XHI'. GIVEN A         *
C * PARTICULAR NUMBER 'Y', THIS SUBROUTINE FINDS THE VALUE OF 'X'       *
C * FOR WHICH 'Y'=POLY(X).  IN OTHER WORDS IT NUMERICALLY SOLVES THE    *
C * EQUATION                                                            *
C *                                                                     *
C *          F(X) = POLY(X) - 'Y' = 0                                   *
C *                                                                     *
C * FOR A UNIQUE SOLUTION TO EXIST, POLY(X) MUST BE MONOTONIC AND 'Y'   *
C * MUST LIE BETWEEN POLY('XLO') AND POLY('XHI').                       *
C *                                                                     *
C * DESCRIPTION OF ALGORITHM                                            *
C * ----------- -- ---------                                            *
C * THE ROOT OF THE POLYNOMIAL LIES ON THE INTERVAL (A,B)=(XLO,XHI).    *
C * THIS MEANS THAT 'F' CHANGES SIGN ONCE ON THE INTERVAL, SO THAT      *
C * F(A)*F(B) < 0.  PICK A POINT MIDWAY BETWEEN A AND B (CALL IT X).    *
C * IF F(A)*F(X) < 0, THE ROOT IS BETWEEN A AND X; B IS THEN ASSIGNED   *
C * THE VALUE OF X. IF F(A)*F(X) > 0, THE ROOT IS BETWEEN X AND B;      *
C * A IS THEN ASSIGNED THE VALUE OF X.  AT THIS POINT WE KNOW THAT THE  *
C * ROOT IS BETWEEN A AND B, BUT THE INTERVAL OF UNCERTAINTY, (B-A),    *
C * IS HALF AS LARGE AS IT WAS INITIALLY.  THE BEST GUESS FOR THE ROOT  *
C * IS (A+B)/2, AND THE MAXIMUM ERROR IN THIS ESTIMATE IS IS (B-A)/2.   *
C * KEEP REPEATING THE ABOVE UNTIL THE ERROR IS SMALLER THAN 'XTOL',    *
C * WHICH HAS BEEN SUPPLIED TO THE SUBROUTINE AS A FORMAL PARAMETER.    *
C *                                                                     *
C * IN SUMMARY, EACH ITERATION HALFS THE MAXIMUM ERROR IN THE ESTIMATE  *
C * OF THE ROOT.  THIS ALGORITHM DOES NOT CONVERGE AS FAST AS SOME      *
C * MORE SOPHISTICATED METHODS (E.G., NEWTON'S METHOD OR MODIFIED       *
C * REGULA FALSI), BUT IT IS GUARANTEED TO FIND THE ROOT. OTHER ALGOR-  *
C * ITHMS ARE SOMETIMES TEMPERAMENTAL.                                  *
C *                                                                     *
C * SUBROUTINE FORMAL PARAMETERS                                        *
C * ----------------------------                                        *
```

```
C * INTEGER                                                              *
C * -------                                                              *
C * IERR - SUBROUTINE ERROR PARAMETER                                    *
C *        1.) SET = 0 IF NO ERRORS ENCOUNTERED                          *
C *        2.) SET = 1 IF NO SOLUTION EXISTS TO EQUATION OVER            *
C *            INTERVAL 'XLO'= 0.0 TO 'XHI'= 1.0                         *
C *        3.) SET = 2 IF NO SOLUTION FOUND TO EQUATION IN MAXIMUM       *
C *            NUMBER OF ITERATIONS ('MAXITR')                           *
C * NDEG - DEGREE OF REGRESSION EQUATION GENERATED BY CALLING            *
C *        PROGRAM 'ASSAY'                                               *
C *                                                                      *
C * DOUBLE PRECISION                                                     *
C * ------ ---------                                                     *
C * Y - INPUT 'UNKNOWN' SAMPLE ABSORBANCE                                *
C * X - OUTPUT 'STANDARD VOLUME EQUIVALENT' (ESTIMATE OF ROOT OF          *
C *     EQUATION AT INPUT 'UNKNOWN' ABSORBANCE).                         *
C * COEFF - VECTOR OF LENGTH 6 CONTAINING REGRESSION COEFFICIENTS        *
C * XLO - LOWER LIMIT OF 'STANDARD VOLUME EQUIVALENT'                    *
C *        SET = TO 0.0                                                  *
C * XHI - UPPER LIMIT OF 'STANDARD VOLUME EQUIVALENT'                    *
C *        SET = TO 1.0                                                  *
C * XTOL - TOLERANCE VALUE FOR MAXIMUM ALLOWED ERROR IN CALCULATED       *
C *        ROOT. SET = TO 1.0E-5                                         *
C *                                                                      *
C * SUBROUTINE SYMBOL DEFINITIONS (EXCLUDING FORMAL PARAMETERS)          *
C * -----------------------------------------------------------          *
C * INTEGER VARIABLES                                                    *
C * ------- ---------                                                    *
C * NDEGP1 - DEGREE OF REGRESSION EQUATION + 1 (LOOP CONTROL)            *
C * MAXITR - MAXIMUM NUMBER OF ITERATIONS ALLOWED TO SOLVE EQUATION      *
C *          WITHIN GIVEN ERROR TOLERANCE                                *
C * ITR - VARIABLE CONTAINING COUNT OF NUMBER OF ITERATIONS COMPLETED    *
C *       BY ROUTINE                                                     *
C *                                                                      *
C * DOUBLE PRECISION VARIABLES                                           *
C * ------ --------- ---------                                           *
C * XERROR - VARIABLE SET = TO XERROR/2 DURING EACH ITERATION  OF        *
C *          SOLUTION ROUTINE. INITIAL VALUE = ABS('XHI'-'XLO'.          *
C * A - TEMP STORAGE VARIABLE. INITIAL VALUE = 'XHI'                     *
C * B - TEMP STORAGE VARIABLE. INITIAL VALUE = 'XLO'                     *
C * POLYA - VARIABLE SET = TO SOLUTION OF POLYNOMIAL AT 'XLO'            *
C * POLYB - VARIABLE SET = TO SOLUTION OF POLYNOMIAL AT 'XHI'            *
C * POLYX - VARIABLE SET = TO SOLUTION OF POLYNOMIAL AT ROOT EST 'X'     *
C * FA - VARIABLE SET = TO 'POLYA - Y'                                   *
C * FB - VARIABLE SET = TO 'POLYB - Y'                                   *
C * FX - VARIABLE SET = TO 'POLYX - Y'                                   *
C *                                                                      *
C ************************************************************************

      SUBROUTINE BSECT (Y,X,COEFF,NDEG,XLO,XHI,XTOL,IERR)
```

```
C SUBROUTINE DATA TYPE STATEMENTS

      INTEGER  NDEG,NDEGP1,IERR,MAXITR,ITR

      DOUBLE PRECISION  Y,X,COEFF(6),XLO,XHI,XTOL,XERROR,A,B,FA,FB,
     1                  FX,POLYA,POLYB,POLYX

C SUBROUTINE SYMBOL INITIALIZATION STATEMENTS

      ITR=0
      IERR=0
      MAXITR=20
      A=XLO
      B=XHI
      XERROR=DABS(B-A)
      NDEGP1=NDEG+1

C EVALUATE POLYNOMIAL GENERATED BY 'ASSAY' USING HORNERS METHOD
C AT 'XLO' AND 'XHI'.

         POLYA=0.
         POLYB=0.
         DO 20 I=1,NDEG
           POLYA=(POLYA+COEFF(NDEGP1-I+1))*A
           POLYB=(POLYB+COEFF(NDEGP1-I+1))*B
20       CONTINUE

         POLYA=POLYA+COEFF(1)
         POLYB=POLYB+COEFF(1)
         FA=POLYA-Y
         FB=POLYB-Y

C CHECK FOR THE EXISTANCE OF A SOLUTION BETWEEN 'XHI' AND
C 'XLO'. IF NO SOLUTION EXISTS SET 'IERR' TO '1' AND RETURN
C TO 'ASSAY'.

         IF(FA*FB.LE.0.) GO TO 30
           IERR=1
           RETURN

30       CONTINUE

C CALCULATE NEW ESTIMATE OF ROOT

         X=(A+B)/2.
         ITR=ITR+1

C CHECK TO SEE IF MAXIMUM NUMBER OF ITERATIONS EXCEEDED.
```

```
      IF(ITR.LE.MAXITR) GO TO 40
        IERR=2
        RETURN

40    CONTINUE

C DIVIDE MAXIMUM ERROR BY 'TWO'.

      XERROR=XERROR/2.

C CHECK TO SEE IF MAXIMUM ERROR IS WITHIN TOLERANCE.

      IF(XERROR.LT.XTOL) RETURN

C EVALUATE POLYNOMIAL AT NEW VALUE OF 'X'.

      POLYX=0.
      DO 50 I=1,NDEG
        POLYX=(POLYX+COEFF(NDEGP1-I+1))*X
50    CONTINUE

      POLYX=POLYX+COEFF(1)
      FX=POLYX-Y

      IF(FA*FX.LE.0.) GO TO 60
        A=X
        FA=FX
        GO TO 30

60    CONTINUE

      B=X
      GO TO 30

   END
```

```
C ***********************************************************************
C *                                                                     *
C * SUBROUTINE 'MONOT'  -  CALLING PROGRAM NAME: 'ASSAY'                *
C *                                                                     *
C * WRITTEN MAY 1981  -  J.J.KNUDSEN, LAIR, DIV.OF RESEARCH SUPPORT,    *
C *                      ANALYTICAL CHEMISTRY GROUP.                    *
C *                                                                     *
C * LANGUAGE  -  DATA GENERAL FORTRAN V                                 *
C *                                                                     *
C * PURPOSE OF SUBROUTINE                                               *
C * ------- -- ----------                                               *
C * THIS SUBROUTINE CHECKS THAT THE REGRESSION POLYNOMIAL IS MONOTONIC *
C * ON (XLO,XHI) BY MAKING SURE THAT THE DERIVATIVE DOES NOT CHANGE    *
C * SIGN FOR 100 POINTS SPACED EVENLY ON THE INTERVAL.  THIS SUB-      *
C * ROUTINE NOT USED IF GENERATED REGRESSION EQUATION IS LINEAR.       *
C *                                                                     *
C * SUBROUTINE FORMAL PARAMETERS                                        *
C * ---------------------------                                         *
C * INTEGER                                                             *
C * -------                                                             *
C * NDEG - DEGREE OF REGRESSION EQUATION GENERATED BY CALLING          *
C *        PROGRAM                                                      *
C * IERROR - SUBROUTINE ERROR PARAMETER                                 *
C *           SET = TO 0 IF 1ST DERIVATIVE OF REGRESSION EQUATION       *
C *           IS MONOTONIC                                              *
C *           SET = TO 1 IF 1ST DERIVATIVE OF REGRESSION EQUATION       *
C *           IS NOT MONOTONIC OVER RANGE 'X'=0.0 TO 1.0                *
C *                                                                     *
C * DOUBLE PRECISION                                                    *
C * ------ ---------                                                    *
C * COEFF - VECTOR OF LENGTH 6 CONTAINING REGRESSION COEFFICIENTS       *
C * XLO - LOWER LIMIT OF 'STANDARD VOLUME EQUIVALENT'                   *
C * XHI - UPPER LIMIT OF 'STANDARD VOLUME EQUIVALENT'                   *
C *                                                                     *
C * SUBROUTINE SYMBOL DEFINITIONS (EXCLUDING FORMAL PARAMETERS)         *
C * -----------------------------------------------------------         *
C * INTEGER VARIABLES                                                   *
C * ------- ---------                                                   *
C * NDEGM1 - DEGREE OF REGRESSION EQUATION - 1 (LOOP CONTROL)           *
C *                                                                     *
C * DOUBLE PRECISION VARIABLES                                          *
C * ------ --------- ---------                                          *
C * DCOEFF - VECTOR OF LENGTH 6 CONTAINING COEFFICIENTS OF 1ST          *
C *          DERIVATIVE OF REGRESSION EQUATION                          *
C * X - VARIABLE SET = TO VALUE BETWEEN 0.0 AND 1.0 AT 100 EQUALLY      *
C *     SPACED POINTS. 1ST DERIVATIVE EVALUATED AT EACH POINT           *
C * DRVX - VALUE OF DERIVATIVE AT 'X'                                   *
C * DRVXLO - VALUE OF DERIVATIVE AT 'XLO'                               *
C * TEST - VARIABLE SET = TO 'DRVX*DRVXLO' TO CHECK IF SIGN OF          *
```

```
C *        DERIVATIVE HAS CHANGED                                       *
C *                                                                      *
C ***********************************************************************

      SUBROUTINE MONOT (COEFF,NDEG,XHI,XLO,IERROR)

C SUBROUTINE DATA TYPE STATEMENTS

      INTEGER  NDEG,NDEGM1,IERROR

      DOUBLE PRECISION  COEFF(6),DCOEFF(6),XLO,XHI,X,DRVX,
     1                  DRVXLO,TEST

C CALCULATE COEFFICIENTS OF 1ST DERIVATIVE OF POLYNOMIAL
C REGRESSION EQUATION GENERATED BY 'ASSAY'

         DO 10 I=1,NDEG
           DCOEFF(I)=COEFF(I+1)*DBLE(FLOAT(I))
10       CONTINUE

C CALCULATE VALUE OF 1ST DERIVATIVE OF REGRESSION EQUATION AT
C 'XLO' USING HORNER'S METHOD.

         NDEGM1=NDEG-1
         DRVXLO=0.

         DO 20 I=1,NDEGM1
           DRVXLO=(DRVXLO+DCOEFF(NDEG-I+1))*XLO
20       CONTINUE

         DRVXLO=DRVXLO+DCOEFF(1)

C CALCULATE VALUE OF 1ST DERIVATIVE OVER RANGE 'XLO' TO 'XHI' AT
C 100 EQUALLY SPACED POINTS. AFTER DERIVATIVE FOR EACH POINT
C CALCULATED, TEST TO DETERMINE IF DERIVATIVE IS POSITIVE
C IF POSITIVE CALCULATE DERIVATIVE AT NEXT POINT.
C IF NEGATIVE SET 'IERROR' TO 1 AND RETURN TO CALLING PROGRAM.

         DO 40 I=1,100
           X=XLO+0.01*I*(XHI-XLO)
           DRVX=0.

           DO 30 J=1,NDEGM1
             DRVX=(DRVX+DCOEFF(NDEG-J+1))*X
30         CONTINUE

           DRVX=DRVX+DCOEFF(1)
           TEST=DRVXLO*DRVX

           IF(TEST.GT.0) GO TO 40
```

```
      IERROR=1
      RETURN
40    CONTINUE
      IERROR=0
      RETURN
      END
```

```
C ************************************************************************
C *                                                                      *
C * SUBROUTINE 'PLOT1'  -  CALLING PROGRAM NAME: 'ASSAY'                 *
C *                                                                      *
C * WRITTEN MAY 1981  -  J.J.KNUDSEN, LAIR, DIV.OF RESEARCH SUPPORT,     *
C *                      ANALYTICAL CHEMISTRY GROUP.                     *
C *                                                                      *
C * LANGUAGE  -  DATA GENERAL FORTRAN V                                  *
C *                                                                      *
C * PURPOSE OF SUBROUTINE                                                *
C * ------- -- ----------                                                *
C * SET UP A TWO DIMENSIONAL ARRAY CALLED 'GRAPH'. THE IMAGE GRID HAS    *
C * 'H' ROWS AND 'W' COLUMNS. THE ARRAY IS INITIALLY FILLED WITH         *
C * BLANKS. THE BOUNDARIES OF THE ARRAY ARE THEN DEFINED WITH APPROP-    *
C * RIATE SYMBOLS AND TICK MARKS AND/OR GRID LINES ADDED.  GRID LINES    *
C * ARE OPTIONAL.                                                        *
C *                                                                      *
C * SUBROUTINE FORMAL PARAMETERS                                         *
C * ---------------------------                                          *
C * INTEGER                                                              *
C * -------                                                              *
C * GRAPH - TWO DIMENSIONAL ARRAY SETUP WITH GRID LINES IN THIS SUB-     *
C *         ROUTINE.                                                     *
C *                                                                      *
C * SUBROUTINE SYMBOL DEFINITIONS (EXCLUDING FORMAL PARAMETERS)          *
C * -------------------------------------------------------------        *
C * INTEGER VARIABLES                                                    *
C * ------- ---------                                                    *
C * W - VARIABLE SET = TO WIDTH OF GRAPH (100 SPACES)                    *
C * H - VARIABLE SET = TO HEIGHT OF GRAPH (50 SPACES)                    *
C * BLANK - VARIABLE SET = TO ALPHAMERIC CHARACTER ' '                   *
C * PLUS - VARIABLE SET = TO ALPHAMERIC CHARACTER '+'                    *
C * MINUS - VARIABLE SET = TO ALPHAMERIC CHARACTER '-'                   *
C * DOT - VARIABLE SET = TO ALPHAMERIC CHARACTER '.'                     *
C * I - VARIABLE USED AS A COUNTER                                       *
C * J - VARIABLE USED AS A COUNTER                                       *
C *                                                                      *
C * SPECIAL INSTRUCTIONS                                                 *
C * ------- ------------                                                 *
C * USE /X SWITCH AT COMPILATION TIME TO DELETE GRID LINES FROM GRAPH    *
C * ARRAY.                                                               *
C *                                                                      *
C ************************************************************************

      SUBROUTINE PLOT1 (GRAPH)

      INTEGER  W,H,GRAPH(0:50,0:100),BLANK,PLUS,MINUS,DOT,I,J

      BLANK=' '
      PLUS='+'
```

```
      MINUS='-'
      DOT='.'

      W=100
      H=50

      J=H
100   I=0
200   GRAPH(J,I)=BLANK
      I=I+1
      IF(I.LE.W) GO TO 200
        J=J-1
      IF(J.GE.0) GO TO 100

      CONTINUE

      J=H
250   K=0
300   I=0
400   GRAPH(J,I+K)=PLUS
      IF(K.EQ.W) GO TO 600
500     I=I+1
X     IF(J.EQ.50.OR.J.EQ.0) GO TO 530
X       GO TO 540
530   GRAPH(J,I+K)=MINUS
540   IF(I.LT.9) GO TO 500
        K=K+10
        GO TO 300
600   IF(J.EQ.0) GO TO 1000
      L=1
620   J=J-1
      K=0
640   I=0

645   CONTINUE

X     IF(I*K.EQ.0.OR.I*K.EQ.100) GO TO 650
X       GO TO 660
650   GRAPH(J,J*K)=DOT
660   IF(I.EQ.10) GO TO 800
        I=I+1
        K=10
        GO TO 645
800   L=L+1
      IF(L.LT.5) GO TO 620
        J=J-1
      IF(J.GE.0) GO TO 250

1000  RETURN
      END
```

```
C ***********************************************************************
C *                                                                     *
C * SUBROUTINE 'PLOT2'  -  CALLING PROGRAM NAME: 'ASSAY'                *
C *                                                                     *
C * WRITTEN MAY 1981  -  J.J.KNUDSEN, LAIR, DIV.OF RESEARCH SUPPORT,    *
C *                      ANALYTICAL CHEMISTRY GROUP.                    *
C *                                                                     *
C * LANGUAGE  -  DATA GENERAL FORTRAN V                                 *
C *                                                                     *
C * PURPOSE OF SUBROUTINE                                               *
C * ------- -- ----------                                               *
C * THIS SUBROUTINE PUTS 'SYMBOL' INTO PLOTTING ARRAY 'GRAPH' CREATED   *
C * BY SUBROUTINE 'PLOT2' AT A POINT CORRESPONDING TO (X,Y) IN THE      *
C * IMAGE PLANE FOR BOTH INPUT RAW DATA FOR STANDARD CURVE AND GEN-     *
C * ERATED REGRESSION EQUATION.  THE RANGE OF COORDINATES IS ASSUMED    *
C * TO BE (XMIN TO XMAX, YMIN TO YMAX) AS DEFINED BELOW.  THE PAIR OF   *
C * REAL COORDINATES (X,Y) IN THE IMAGE PLANE ARE CONVERTED TO A PAIR   *
C * OF INTEGER SUBSCRIPTS (I,J) USING THE DISCRETIZATION FORMULA:       *
C *                                                                     *
C *          I = (X - XMIN/XMAX - XMIN) * N                             *
C *          J - (Y - YMIN/YMAX - YMIN) * N                             *
C *                                                                     *
C *  WHERE: N = DESIRED NUMBER OF DISCRETIZATION INTERVALS              *
C *                                                                     *
C * SUBROUTINE FORMAL PARAMETERS                                        *
C * ---------------------------                                         *
C * INTEGER                                                             *
C * -------                                                             *
C * GRAPH - TWO DIMENSIONAL ARRAY SET UP BY 'PLOT1'. VALUES OF INPUT    *
C *         STANDARD CURVE DATA POINTS INSERTED IN THIS ARRAY AS WELL   *
C *         AS 50 EQUALLY SPACED POINTS OF REGRESSION EQUATION GEN-     *
C *         ERATED BY 'ASSAY'.                                          *
C * N - NUMBER OF STANDARD CURVE DATA POINTS ENTERED IN MAIN PROGRAM    *
C * NDEG - DEGREE OF REGRESSION EQUATION GENERATED BY 'ASSAY'           *
C * A - VARIABLE SET = TO 0 IF 'MAXABS' IN MAIN PROGRAM <= 1.0          *
C *              SET = TO 1 IF 'MAXABS' IN MAIN PROGRAM >  1.0          *
C * REAL                                                                *
C * ----                                                                *
C * DAT - TWO DIMENSIONAL ARRAY CONTAINING STANDARD CURVE DATA POINTS   *
C *                                                                     *
C * DOUBLE PRECISION                                                    *
C * ------ ---------                                                    *
C * COEFF - VECTOR OF LENGTH 6 CONTAINING REGRESSION COEFFICIENTS       *
C *                                                                     *
C * SUBROUTINE SYMBOL DEFINITIONS (EXCLUDING FORMAL PARAMETERS)         *
C * ----------------------------------------------------------         *
C * INTEGER VARIABLES                                                   *
C * ------- ---------                                                   *
C * SYMBOL - VARIABLE CONTAINING SYMBOL TO BE USED IN PLOTTING INPUT    *
```

```
C *          STD CURVE DATA POINTS AND REGRESSION EQUATION POINTS        *
C *           SET = TO 'O' FOR STD CURVE DATA POINTS                     *
C *           SET = TO '@' FOR REGRESSION EQUATION DATA POINTS           *
C * W - WIDTH OF ARRAY 'GRAPH'                                           *
C * H - HEIGTH OF ARRAY 'GRAPH'                                          *
C * I - VARIABLE TO CONTAIN 'X-AXIS' INFORMATION FOR PLOTTING            *
C * J - VARIABLE TO CONTAIN 'Y-AXIS' INFORMATION FOR PLOTTING            *
C * NDEGP1 - DEGREE OF REGRESSION EQUATION + 1 (LOOP CONTROL)            *
C *                                                                      *
C * DOUBLE PRECISION VARIABLES                                           *
C * ------ --------- ---------                                           *
C * XLO - LOWER LIMIT OF 'STANDARD VOLUME EQUIVALENT'                    *
C * XHI - UPPER LIMIT OF 'STANDARD VOLUME EQUIVALENT'                    *
C * X - VARIABLE USED IN SOLUTION OF REGRESSION EQUATION FOR PLOTTING    *
C *     PURPOSES. 'X-AXIS' INFORMATION.                                  *
C * Y - VARIABLE USED IN SOULTION OF REGRESSION EQUATION FOR PLOTTING    *
C *     PURPOSES. 'Y-AXIS' INFORMATION.                                  *
C * XMIN - MINIMUM VALUE OF 'X'.                                         *
C * XMAX - MAXIMUM VALUE OF 'X'.                                         *
C * YMIN - MINIMUM VALUE OF 'Y'.                                         *
C * YMAX - MAXIMUM VALUE OF 'Y'. SET = TO 1.0 IF A = 0, SET = TO         *
C *        2.0 IF A = 1.                                                 *
C *                                                                      *
C ************************************************************************

      SUBROUTINE PLOT2 (GRAPH,N,DAT,NDEG,COEFF,A)

      INTEGER  GRAPH(0:50,0:100),SYMBOL,I,J,W,H,NDEG,NDEGP1,N,A

      REAL  DAT(30,3)

      DOUBLE PRECISION  XHI,XLO,COEFF(6),X,Y,XMIN,XMAX,YMIN,YMAX

      IF(A.EQ.0) GO TO 20
        YMAX=2.0
        GO TO 30
20    YMAX=1.0
30    XMAX=1.0
      YMIN=0.0
      XMIN=0.0
      XLO=0.0
      XHI=1.0

      H=51
      W=100

         SYMBOL='O'
         DO 100 K=1,N
           I=INT(DAT(K,3)*(DBLE(FLOAT(H))))
           J=INT(((DAT(K,2)-YMIN)/(YMAX-YMIN))*(DBLE(FLOAT(W))))
```

```
            I=50-I

            IF((I.GE.0) .AND.
     1         (I.LE.H) .AND.
     1         (J.GE.0) .AND.
     1         (J.LE.W)       ) GO TO 75
            GO TO 100
75          GRAPH(I,J)=SYMBOL

100       CONTINUE

          SYMBOL='@'
          NDEGP1=NDEG+1
          DO 200 K=0,50
            X=XLO+0.02*K*(XHI-XLO)

            Y=0.
            DO 150 L=1,NDEG
              Y=(Y+COEFF(NDEGP1-L+1))*X
150         CONTINUE

            Y=Y+COEFF(1)

            I=INT(X*(DBLE(FLOAT(H))))
            J=INT(((Y-YMIN)/(YMAX-YMIN))*(DBLE(FLOAT(W))))
            I=50-I

            IF((I.GE.0) .AND.
     1         (I.LE.H) .AND.
     1         (J.GE.0) .AND.
     1         (J.LE.W)       ) GO TO 175
            GO TO 200
175         GRAPH(I,J)=SYMBOL


200       CONTINUE

          RETURN

          END
```

*** STATISTICAL DATA --> CALCULATED POLYNOMIAL REGRESSION EQUATION ***

REQUESTED DEGREE OF REGRESSION EQUATION: 1
DEGREE OF CALCULATED REGRESSION EQUATION: 1

** ANALYSIS OF VARIANCE **

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUMS OF SQUARES	MEAN SQUARES	COMPUTED F-VALUE	TAIL AREA F-DISTRIBUTION
REGRESSION	1.	1.07912	1.07912	21072.054	.000000060
RESIDUAL	4.	.00020	.00005		
CORRECTED TOTAL	5.	1.07933			

** REGRESSION COEFFICIENTS **

DEGREE OF TERM	VARIABLE MEANS	REGRESSION COEFFICIENTS	STANDARD ERROR	LOWER CONFIDENCE LIMIT	UPPER CONFIDENCE LIMIT	ADJUSTED SUMS OF SQUARES	PARTIAL F-TEST VALUES	TAIL AREA F-DISTRIBUTION
X**1	.50000	1.24161436	.00855	1.2179	1.2654	1.07912	********	.999999940
INTERCEPT:	.63432	.01350950	.00518	-.0009	.0279	.00000	.0000	.000000000

PERCENTAGE OF VARIATION EXPLAINED BY ESTIMATED MODEL: 99.981021
STANDARD DEVIATION OF RESIDUALS: .007156

POLYNOMIAL CALCULATION SUBROUTINE ERROR PARAMETER: 0

APPENDIX C

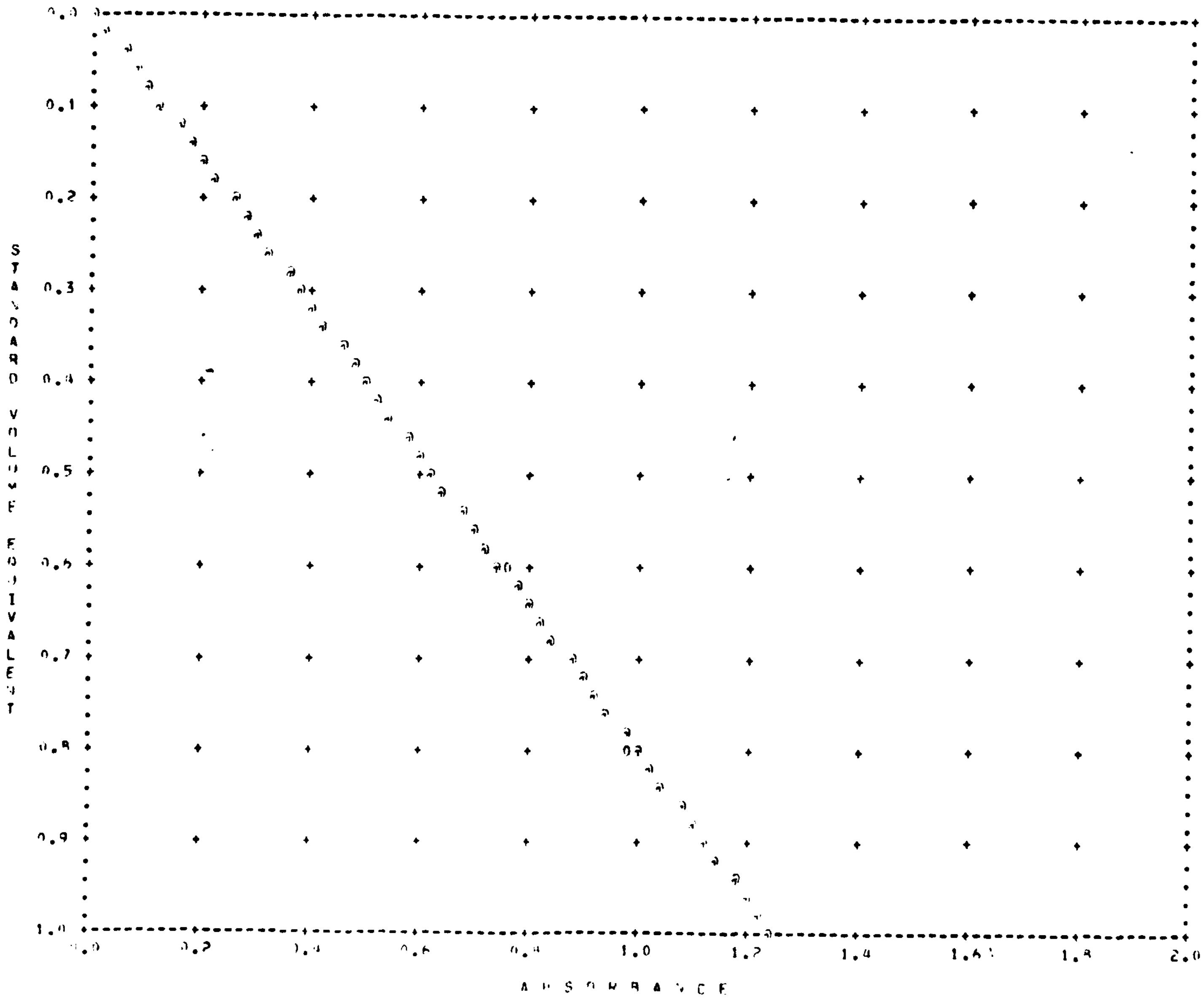
*** INPUT DATA AND PREDICTED VALUES FROM MODEL ***

DATA POINT NUMBER	-X- INPUT STANDARD VOLUME	-X- SCALED STANDARD EQ. VOLUME	-Y- INPUT ABSORBANCE	-Y- PREDICTED ABSORBANCE	RESIDUAL VALUE
1	.0000	.0000	.0188	.0135	.00529
2	.1000	.2000	.2609	.2618	-.00093
3	.2000	.4000	.5043	.5102	-.00586
4	.3000	.6000	.7601	.7585	.00162
5	.4000	.8000	.9984	1.0068	-.00840
6	.5000	1.0000	1.2634	1.2551	.00828

X --> INDEPENDENT VARIABLE
Y --> DEPENDENT VARIABLE

*** CALCULATED REGRESSION EQUATION - LOW RESOLUTION PLOT - (X-AXIS VERTICAL; Y-AXIS HORIZONTAL) ***

SYMBOL FOR INPUT DATA POINTS: => 0
SYMBOL FOR REGRESSION EQUATION: => @



ASSAY RESPONSE PROGRAM - SAMPLE DATA OUTPUT

TYPE OF ASSAY: [illegible]
DATE OF ASSAY: [illegible] JULY 1981
SAMPLE TYPE: KALAKRIE[illegible] - ENTERO
NAME OF INVESTIGATOR: TRAVERSO

ASSAY PARAMETERS => STANDARD CONCENTRATION: 30.00 U/ML
SAMPLE DILUTION FACTOR: 2.00
NO. OF SAMPLE DILUTIONS: 1
SAMPLE CONCENTRATION UNITS: U/ML

SAMPLE DILUTION 1 = .500 ML

SAMPLE NO.	ABSORBANCE DIL 1	ABSORBANCE DIL 2	ABSORBANCE DIL 3	ABSORBANCE DIL 4	CONCENTRATION DIL 1	CONCENTRATION DIL 2	CONCENTRATION DIL 3	CONCENTRATION DIL 4	MEAN CONC (MC)	ADJUSTED CONCENTRATION: NO. OF DIL VOLS EXCEEDING +/-(.10*MC + MC)	ADJUSTED CONCENTRATION: ADJUSTED MEAN CONC	NO. IN AVG.
101	1.176	.000	.000	.000	56.2	.0	.0	.0	56.2 [	0.	56.2	1.]
102	1.162	.000	.000	.000	55.5	.0	.0	.0	55.5 [	0.	55.5	1.]
103	1.046	.000	.000	.000	49.9	.0	.0	.0	49.9 [	0.	49.9	1.]
104	.622	.000	.000	.000	29.4	.0	.0	.0	29.4 [	0.	29.4	1.]
105	.019	.000	.000	.000	-111.0	.0	.0	.0	.0 [	1.	-333.0	0.]

ERROR CODES - CONCENTRATION CALCULATION ROUTINE

1.) -111. IN CONCENTRATION COLUMNS INDICATES THAT SAMPLE ABSORBANCES NOT IN RANGE OF STANDARD CURVE.

2.) -333. IN ADJUSTED CONCENTRATION COLUMNS INDICATES THAT THE CONCENTRATION OF UNKNOWN AT ALL DILUTIONS EXCEEDED -> +/-(.10*MC + MC).

*** STATISTICAL DATA --> CALCULATED POLYNOMIAL REGRESSION EQUATION ***

REQUESTED DEGREE OF REGRESSION EQUATION: 4
DEGREE OF CALCULATED REGRESSION EQUATION: 2

** ANALYSIS OF VARIANCE **

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUMS OF SQUARES	MEAN SQUARES	COMPUTED F-VALUE	TAIL AREA F-DISTRIBUTION
REGRESSION	2.	.83802	.41901	2672.979	.000000000
RESIDUAL	15.	.00235	.00016		
CORRECTED TOTAL	17.	.84037			

** REGRESSION COEFFICIENTS **

DEGREE OF TERM	VARIABLE MEANS	REGRESSION COEFFICIENTS	STANDARD ERROR	LOWER CONFIDENCE LIMIT	UPPER CONFIDENCE LIMIT	ADJUSTED SUMS OF SQUARES	PARTIAL F-TEST VALUES	TAIL AREA F-DISTRIBUTION
X**1	.38333	.94569192	.03278	.8758	1.0156	.13043	832.0737	.999999940
X**2	.25583	-.30811186	.03269	-.3778	-.2384	.01392	88.8243	.999999891
INTERCEPT:	.54989	.06619894	.00586	.0537	.0787	.00000	.0000	.000000000

PERCENTAGE OF VARIATION EXPLAINED BY ESTIMATED MODEL: 99.720199
STANDARD DEVIATION OF RESIDUALS: .012520

POLYNOMIAL CALCULATION SUBROUTINE ERROR PARAMETER: 0

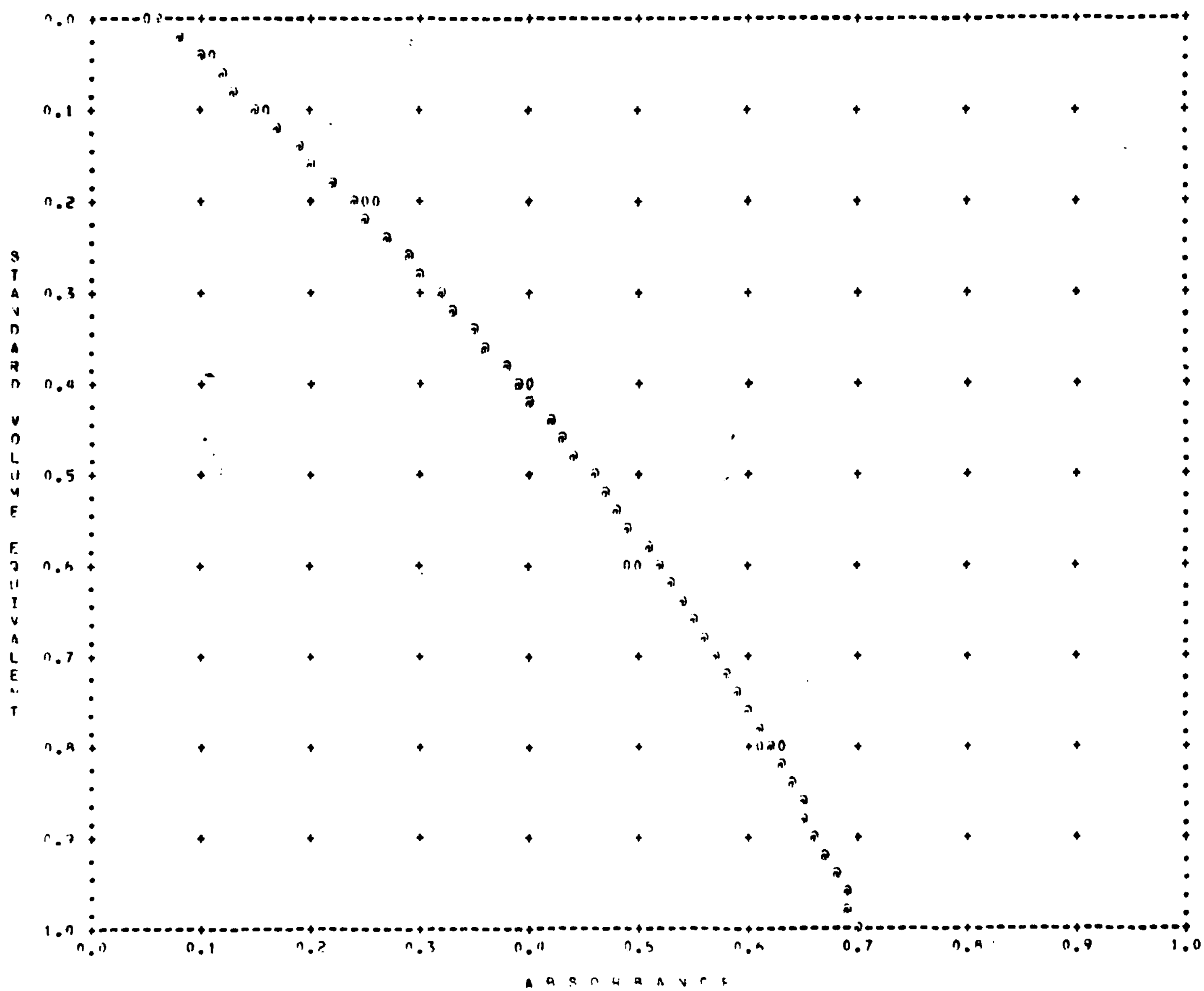
*** INPUT DATA AND PREDICTED VALUES FROM MODEL ***

DATA POINT NUMBER	-X- INPUT STANDARD VOLUME	-X- SCALED STANDARD EQ. VOLUME	-Y- INPUT ABSORBANCE	-Y- PREDICTED ABSORBANCE	RESIDUAL VALUE
1	.0000	.0000	.0550	.0662	-.01120
2	.0000	.0000	.0540	.0662	-.01220
3	.0500	.0500	.1030	.1127	-.00371
4	.0500	.0500	.1120	.1127	-.00071
5	.1000	.1000	.1610	.1577	.00331
6	.1000	.1000	.1610	.1577	.00331
7	.2000	.2000	.2550	.2430	.01199
8	.2000	.2000	.2620	.2430	.01899
9	.3000	.3000	.3250	.3222	.00282
10	.3000	.3000	.3280	.3222	.00582
11	.4000	.4000	.4040	.3952	.00882
12	.4000	.4000	.3990	.3952	.00382
13	.6000	.6000	.5100	.5227	-.01269
14	.6000	.6000	.4950	.5227	-.02769
15	.8000	.8000	.6380	.6256	.01244
16	.8000	.8000	.6100	.6256	-.01556
17	1.0000	1.0000	.7100	.7038	.00622
18	1.0000	1.0000	.7100	.7038	.00622

X --> INDEPENDENT VARIABLE
Y --> DEPENDENT VARIABLE

*** CALCULATED REGRESSION EQUATION - LOW RESOLUTION PLOT - [X-AXIS VERTICAL; Y-AXIS HORIZONTAL] ***

SYMBOL FOR INPUT DATA POINTS: => 0
SYMBOL FOR REGRESSION EQUATION: => @



ASSAY REGRESSION PROGRAM - SAMPLE DATA OUTPUT

TYPE OF ASSAY: VITAMIN B-6
DATE OF ASSAY: 19 MARCH 1980
SAMPLE TYPE: CHICKEN RAD FOOD
NAME OF INVESTIGATOR: MCGOWN

ASSAY PARAMETERS => STANDARD CONCENTRATION: 4.00 NG/ML
SAMPLE DILUTION FACTOR: 250.00
NO. OF SAMPLE DILUTIONS: 4
SAMPLE CONCENTRATION UNITS: NG/ML

SAMPLE DILUTION 1 = .030 ML
SAMPLE DILUTION 2 = .050 ML
SAMPLE DILUTION 3 = .100 ML
SAMPLE DILUTION 4 = .200 ML

SAMPLE NO.	ABSORBANCE				CONCENTRATION				MEAN CONC (MC)	ADJUSTED CONCENTRATION: NO. OF DIL VOLS EXCEEDING +/-(.10*MC + MC)	ADJUSTED MEAN CONC	NO. IN AVG.
	DIL 1	DIL 2	DIL 3	DIL 4	DIL 1	DIL 2	DIL 3	DIL 4				
1	.094	.137	.212	.320	989.5	1535.8	1628.0	1485.7	1409.8	2.	1510.6	2.
2	.092	.145	.206	.306	917.8	1714.3	1557.2	1394.6	1396.0	3.	1394.6	1.
3	.117	.180	.263	.404	1823.2	2509.3	2245.3	2063.4	2160.3	2.	2150.3	2.
4	.115	.184	.293	.399	1749.9	2601.5	2622.3	2027.4	2250.3	3.	2027.4	1.
5	.085	.114	.133	.000	667.1	1028.3	723.5	.0	806.3	4.	-333.0	0.
6	.085	.115	.164	.212	667.1	1050.0	1071.5	814.0	900.6	3.	814.0	1.
7	.087	.128	.184	.247	738.3	1336.2	1300.7	1024.3	1099.9	3.	1024.3	1.
8	.092	.127	.178	.255	917.8	1313.9	1231.6	1073.3	1134.2	2.	1152.4	2.
9	.144	.215	.357	.000	2820.1	3327.2	3466.6	.0	3204.6	2.	3396.9	2.
10	.130	.202	.301	.429	2300.8	3020.5	2724.7	2247.3	2573.3	3.	2724.7	1.

ERROR CODES - CONCENTRATION CALCULATION ROUTINE

1.) -111. IN CONCENTRATION COLUMNS INDICATES THAT SAMPLE ABSORBANCES NOT IN RANGE OF STANDARD CURVE.

2.) -333. IN ADJUSTED CONCENTRATION COLUMNS INDICATES THAT THE CONCENTRATION OF UNKNOWN AT ALL DILUTIONS EXCEEDED => +/-(.10*MC + MC).

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